PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
A01N 33/02, 33/04	A1	WO 93/00811
		(43) International Publication Date: 21 January 1993 (21.01.93)

(21) International Application Number: PCT/US92/05473

(22) International Filing Date: 30 June 1992 (30.06.92)

(30) Priority data:

721,322	1 July 1991 (01.07.91)	US
814,877	2 January 1992 (02.01.92)	US

(71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; Fruit Street, Boston, MA 02114 (US).

(72) Inventor: NATHANSON, James, A. ; 1 Grove Street, Wellesley, MA 02181 (US).

(74) Agents: GOLDSTEIN, Jorge, A. et al.; Sterne, Kessler, Goldstein & Fox, 1225 Connecticut Avenue, N.W., Suite 300, Washington, DC 20036 (US).

(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE).

Published
With international search report.

(54) Title: INVERTEBRATE PHENYLETHANOLAMINE TRANSPORTER AND THE USE THEREOF

(57) Abstract

A method of controlling an invertebrate pest, comprising contacting the pest with a pest-controlling amount of an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive octopamine reuptake inhibition assay is disclosed. Compositions comprising compounds capable of inhibiting the octopamine reuptake transporter include chloroethylphenylamines, aryl-1,4-dialy(en)yl piperazines, tricyclic antidepressants, and cocaine derivatives. A process for inhibiting the feeding of an invertebrate pest comprising contacting said pest with a pest-controlling amount of an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive octopamine reuptake inhibition assay, with the proviso that said agent is not cocaine. A process for delaying the maturation of a juvenile invertebrate by contacting it with an inhibitory amount of a phenylethanolamine reuptake transporter blocker is also disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland			SN	Senegal
CI	Côte d'Ivoire	KR	Republic of Korea	SU	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
CS	Czechoslovakia	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		
ES	Spain	MG	Madagascar		

Title of the Invention

INVERTEBRATE PHENYLETHANOLAMINE TRANSPORTER AND THE USE THEREOF

Cross Reference to Related Applications

The present application is a continuation-in-part of U.S. Patent Application Serial No. 07/721,322, filed July 1, 1991, the contents of which are fully incorporated herein by reference.

Field of the Invention

The present invention is in the field of pest-controlling agents. In particular, the invention relates to a method for inhibiting an invertebrate-specific membrane transporter protein, compounds having binding specificity therefor, and pesticidal/pestistatic compositions.

Background of the Invention

A. Octopamine and Octopamine Receptors.

Octopamine (OA) was first discovered over 35 years ago in the posterior salivary gland of the octopus (V. Erspamer and G. Boretti, *Arch. Int. Pharmaco. Ther. 88:*926-322 (1951)). Although similar to norepinephrine (NE) in structure, OA has very little activity as a sympathomimetic when injected into mammals (A. Lands and J. Grant, *J. Pharm. Exptl. Therap. 106:*341-345 (1952)) and, compared with NE, is present in very low concentrations in vertebrate tissues (Y. Kakimoto and M. Armstrong, *J. Biol. Chem. 237:*422-427 (1962)). Relatively little attention was paid to OA until the early 1970's, when Molinoff & Axelrod reported that OA was present in much higher concentrations in invertebrates, particularly in invertebrate nerve tissue (P.B. Molinoff and J. Axelrod, *J. Neurochem. 19:*157-163 (1972)).

In 1973, the first identification of an OA receptor was reported (J.A. Nathanson "*Cyclic AMP: A Possible Role in Insect Nervous System Function*", (Ph.D. Thesis) (1973); J.A. Nathanson and P. Greengard,

*Science, 19:*308-310 (1973)). Because this receptor was present in highest concentrations in insect nerve cord, it was postulated that OA might function as a neurotransmitter. Furthermore, because these receptors were undetectable in mammalian tissues, it was also postulated that the neurotransmitter function of OA might be largely restricted to invertebrates (J.A. Nathanson "*Cyclic AMP: A Possible Role in Insect Nervous System Function*", (Ph.D. Thesis) (1973); J.A. Nathanson and P. Greengard, *Science, 19:*308-310 (1973); J.A. Nathanson, *Trace Amines and the Brain; Eds. Marcel Dekker*, pp. 161-190 (1976)). At about the same time, Kravitz and coworkers (B. Wallace *et al., Brain Res.* 349-55.16 (1974)) independently reported the presence of OA-containing neurons in crustacea, and, somewhat later, Hoyle reported evidence suggesting the presence of large OA neurons in insect ganglia (G. Hoyle, *J. Exp. Zool 193:*425-31 (1975)). Subsequent work by a number of investigators has established the role of OA, not only as a neurotransmitter, but also as a neuromodulator and circulating neurohormone in insects and acarines (for review see I. Orchard, *Can. J. Zool, 60:*659-69 (1982); H.A. Robertson and A.V. Juorio, *Int. Rev. Neurobiol. 19:*173-224 (1976)). Indeed, OA plays a pervasive role in regulating many areas of insect physiology, including carbohydrate metabolism, lipid mobilization, hematocyte function, heart rate, peripheral muscle tension and excitability, and behavior. The functions that OA carries out in insects appear analogous to those carried out by norepinephrine (NE) and epinephrine (EPI) in vertebrates. This has led to the suggestion that, during evolution, there may have been a divergence in the use of these amines between the two arms of the animal kingdom (H.A. Robertson and A.V. Juorio, *Int. Rev. Neurobiol. 19:*173-224 (1976); A.V. Robertson and A.V. Juorio, *J. Neurochem. 28:*573-79 (1977); J.A. Nathanson, *Physiological Reviews 57:*158-256 (1977)).

Analogous to the action of NE and EPI in vertebrates, many of the effects of OA in invertebrates are mediated by cyclic AMP (J.A. Nathanson and P. Greengard, *Science 19:*308-310 (1973); J.A. Nathanson,

*Physiological Reviews 57:*158-256 (1977); H. Robertson and J. Steele, *J. Neurochem 19:*1603-06 (1972); A. Harmar and A. Horn, *Mol. Pharmacol. 13:*512-20 (1976); C. Lingle *et al., Handbook of Exptl. Pharmacology, (J. Kebabian & J. Nathanson, eds.)*, pp. 787-846 (1982)). OA stimulates production of cyclic AMP through activation of OA-sensitive (G_s protein-coupled) adenylate cyclase (J.A. Nathanson, *J. Cyclic Nucleotide and Protein Phosphor. Res. 10:*157-66 (1985)). In 1979, it was found that the firefly light organ, in which OA mediates neural control of light emission (A.D. Carlson, *Advances Insect Physiol. 6:*51-96 (1969); J.F. Case and L.G. Strause, *Bioluminescence in Action (P.J. Herring, ed.)*, pp. 331-366 (1978)), has a virtually pure population of OA receptors present in enormous quantity, with no evidence of adenylate cyclases activated by other hormones (J.A. Nathanson, *Science 203:*65-8 (1979); J.A. Nathanson and E. Hunnicutt, *J. Exp. Zool. 208:*255-62 (1979a)). Thereafter, the first detailed pharmacological characterization of G_s-linked OA receptors was carried out in the absence of other amine receptors (J.A. Nathanson, *Science 203:*65-8 (1979); J.A. Nathanson and E. Hunnicutt, *J. Exp. Zool. 208:*255-62 (1979a); J.A. Nathanson, *Proc. Natl. Acad. Sci. USA 82:*599-603 (1985b);Nathanson, J.A., in *Insect Neurochemistry and Neurophysiology*, Borkovec, A., *et al.*, eds., Humana: Clifton, NJ, pp. 263-266 (1986); Nathanson, J.A., *et al., Neurosci. Abstr. 5:*346 (1979)). More recently, a new chemical class of potent OA receptor agonists has been characterized, the phenyliminoimidazolidines (PIIs) (Nathanson, J.A., *Proc. Natl. Acad. Sci. USA 82:*599-603 (1985); Nathanson, J.A., *Mol. Pharmacol. 28:*254-268 (1985)). With the PIIs and other compounds, it has been possible to distinguish clearly the characteristics of OA receptors from those of mammalian adrenergic, dopaminergic, and serotonergic receptors.

Overactivation of the OA system in insects and acarines leads to behavioral and physiological abnormalities that have pestistatic and pesticidal consequences. One way to cause OA overactivation, and

thereby take advantage of this system for pesticide development, is to directly stimulate OA receptor proteins.

Analogous to the octopamine neurotransmitter system is the cholinergic system, where the plant alkaloid nicotine exerts natural pesticidal effects through excessive activation of acetylcholine (ACh) receptors. As is well known, for pesticide development it has turned out that, more effective than cholinergic agonists, are the reversible and irreversible acetylcholinesterase inhibitors. Acetylcholinesterase (AChE) catalyzes the hydrolysis of the neurotransmitter ACh to choline and acetate. If AChE is inhibited by a pesticide, normal inactivation of ACh is blocked, and ACh accumulates to abnormally high levels. This causes overactivation of ACh receptors, indirectly, through inhibition of neurotransmitter degradation. We have recently discovered that an analogous site of action exists for the OA system. Because of OA's selectivity for invertebrates, agents affecting this site will have reduced toxicity for vertebrates.

B. Pesticidal and Pestistatic Activity of OA Agonists in Insects and Acarinas.

Because OA affects so many sites in insects, it is not surprising that disruption of this system adversely affects insect physiology. In 1980, it was reported that the formamidine pesticides cause glowing of firefly light organs and it was suggested that these compounds, which have low toxicity for vertebrates, might be exerting their pesticidal actions by affecting OA receptors. Subsequently, several labs (Nathanson, J.A., *et al.*, *Molec. Pharmacol. 20*:68-75 (1981); Evans, P.D., *et al.*, *Nature 287*:60-62 (1980)), determined that the formamidines are indeed potent OA agonists in several insect species. In addition, it was found that OA itself, as well as OA analogs and the PIIs applied to leaves, could markedly interfere with the feeding of *M. sexta* (Nathanson, J.A., *Proc. Natl. Acad. Sci. USA 82*:599-603 (1985); Nathanson, J.A., in *Insect Neurochemistry and*

Neurophysiology, Borkovec, A., *et al.*, eds., Humana: Clifton, NJ, pp. 263-266 (1986); Nathanson, J.A., *Mol. Pharmacol. 28*:254-268 (1985); Nathanson, J.A., in *Sites of Action for Neurotoxic Pesticides*, Hollingworth, R., *et al.*, eds., Am. Chem. Soc.: Washington, DC, pp. 154-161 (1987); Nathanson, J.A., *Science 226*:184-187 (1984); Nathanson, J.A., in *Abstr. 2nd Internatl. Symp. Insect Neurobiol. Pest. Action*, Society of Chemical Industry: London, pp. 129-130 (1985); Nathanson, J.A., in *Membrane Receptors and Enzyme as Targets of Insecticidal Action*, Clark, J., *et al.*, Plenum: New York, pp. 157-171 (1986)). The behavioral and pestistatic effects of these compounds on *Manduca* were similar to those of the formamidines: they caused tremors, hyperactivity, rearing, and poor coordination (resulting in leaf drop-off), abnormalities which, interestingly, are reminiscent of the effects of overdoses of amphetamines and adrenergic agonists in vertebrates.

Additional support for a connection between overactivation of the OA system and pesticidal activity has come from observations showing that the known species variation in the pesticidal effects of formamidines (Matsumura, F., *et al.*, *Environ. Health Perspect. 14*:71-82 (1976)) is related to the ability of these compounds to activate G_s-coupled OA receptors (G_s-coupled receptors are those whose activation results in the stimulation of adenylate cyclase and the synthesis of cyclic AMP). For example, in *Manduca*, a sensitive species, we have found (Nathanson, J.A., *Mol. Pharmacol. 28*:254-268 (1985)) that didemethylchlordimeform (DDCDM) is a full OA agonist, 20-fold more potent than OA, while in cockroach, a resistant species, DDCDM is much weaker than OA in activating adenylate cyclase. This species variability appears to result from the distribution of OA receptor subtypes that need to be specifically targeted for pesticide activity. (Additional evidence for involvement of G_s (cAMP-linked) OA receptors comes from the observations (Nathanson, J.A., *Proc.*

Natl. Acad. Sci. USA 82:599-603 (1985)) that the antifeeding effects of OA agonists are enhanced by inhibitors of cAMP catabolism and mimicked by adenylate cyclase activators (forskolin) and lipid-soluble cAMP analogs.)

C. Cocaine and the Amine Reuptake Site.

Although cocaine has a fascinating medicinal history in man, dating back at least 4500 years, its natural function in plants is unknown (Plowman, T., in *Ethnobotany in the Neotropics*, Prance, G.T., *et al.*, eds., N.Y. Botanical Garden, Bronx, NY, pp. 62-111 (1984)). Plowman, Rivier and others (Rivier, L., *J. Ethnopharmacology 3*:313-335 (1981); Plowman, T., *et al.*, *Ann. Bot. (London) 51*:641-659 (1983)) have determined that the four major varieties of *Erythroxylum* (coca) plants that produce cocaine, contain levels ranging from 0.35 - 0.72% dry weight, with values often exceeding 1% (particularly in small, newly emerging leaves). Although relatively little is known about the insect pests of coca, Plowman & Well (*J. Ethnopharmacology 1*:263-278 (1979)) have commented, on the basis of personal observations, that:

> Compared with other tropical American crops, *E. coca* and *E. novogranatense* are relatively pest-free. Herbivorous insects are only rarely observed on the plants in the field; damage to leaves is often minor. This is especially noteworthy since, during much of the year, the membranaceous leaves of coca are found in the tender state of unfolding, the result of their being stripped 3-6 times a year during harvest.

D. Amine Reuptake in The Adrenergic System.

In sympathetic neuroeffector junctions, termination of norepinephrine (NE) action is effected by active reuptake into the nerve by a membrane-bound amine reuptake transport system. This process is largely responsible for the termination of the effects of adrenergic impulses in most organs. To effect the reuptake of norepinephrine into adrenergic nerve terminals and to maintain the concentration gradient of

norepinephrine within the vesicles, at least two distinct carrier-mediated transport systems are involved: one across the axoplasmic membrane from the extracellular fluid to the cytoplasm; and the other from the cytoplasm into the storage vesicles.

Due to the relative ease of isolating pure preparations of granules, especially from the adrenal medulla, the transport system of the storage granule has been well-characterized. It can concentrate catecholamines against a 200-fold gradient across the membrane of the chromaffin granule. This transport system requires ATP and Mg^{2+}, and it is blocked by low concentrations (40 nM) of reserpine. Uptake of catecholamine and ATP into isolated chromaffin granules appears to be driven by pH and potential gradients that are established by an ATP-dependent proton translocase (Winkler *et al.*, Molecular organization of vesicles storing transmitter: chromaffin vesicles as a model. In, *Chemical Neurotransmission* - 75 years, (Stjärne *et al.*, eds.) Academic Press, Ltd., London, 1981, pp. 57-68).

The amine transport system across the axoplasmic membrane is Na^+ dependent and is blocked by a number of drugs, including cocaine and the tricyclic antidepressants, such as imipramine. This transporter has a high affinity for norepinephrine and a somewhat lower affinity for epinephrine; the synthetic β-adrenergic agonist isoproterenol is not a substrate for this system. The neuronal uptake process has been termed *uptake-1* (Iversen, L.L., Uptake processes for biogenic amines. In, *Handbook of Psychopharmacology*, Vol. 3 (Iversen *et al.*, eds.) Plenum Press, New York, pp. 381-442, 1975). There is also an extraneuronal amine transport system, termed *uptake-2*, which exhibits a low affinity for norepinephrine, a somewhat higher affinity for epinephrine, and a still higher affinity for isoproterenol. This uptake process is quite ubiquitous and is present in glial, hepatic, myocardial, and other cells. Uptake-2 is not inhibited by imipramine or cocaine. It is probably of relatively little physiological importance unless the neuronal uptake mechanism is

blocked (Iversen, 1975, *Id.*; Trendelenburg, U., A kinetic analysis of the extraneuronal uptake and metabolism of catecholamines, *Rev. Physiol. Biochem. Pharmacol.*, *87*:33-115 (1980)). It may be of greater importance in the disposition of circulating catecholamines than in the removal of amines that have been released from adrenergic nerve terminals.

In mammals, the CNS stimulatory and euphoric effects of cocaine are thought to be due to cocaine's well-documented action in blocking dopamine (DA) reuptake into presynaptic nerve terminals (Jaffe, J., in *The Pharmacological Basis of Therapeutics*, Gilman, A.G., *et al.*, eds., MacMillan: New York, pp. 535-584 (1980); Kennedy, L., *et al.*, *J. Neurochem. 41*:172-178 (1983)). Because amine reuptake is a major mechanism for inactivation of DA following its synaptic release, the effect of cocaine is to augment and prolong DA neurotransmission. In man, with moderate amounts of cocaine, this results in mild verbal and motor activation which is reinforcing but in excess cocaine may cause hyperactivity, tremors, incoordination, vomiting, and tonic-clonic convulsions.

Pharmacological evidence from vertebrates supports the presence of distinct reuptake sites (amine transporter proteins) for DA, NE, and serotonin (5-HT) (Ritz, M., *et al.*, *NIDA Research Monograph 95*:239-246 (1989)). Although much recent emphasis has been put on cocaine's action on DA, older literature clearly indicates that cocaine also blocks the reuptake of NE and 5-HT (Baldessarini, R., *et al.*, *J. Neurochem. 18*:2519-2533 (1971); Body, T., *et al.*, *Pharmacol. Biochem. & Behavior 34*:165-172 (1989)).

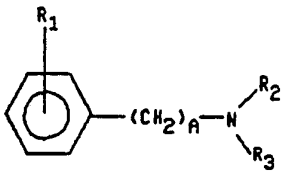
Summary of the Invention

Octopamine (OA) is an invertebrate-specific neurotransmitter. The question of whether there might be some way to create a more general overactivation of the OA system, coupled with our curiosity about the role of cocaine in plants, has led us to discover a new site for pesticide action.

It has been discovered that when the invertebrate octopamine reuptake transporter is blocked by several classes of compounds, herein described, the result is pest-controlling activity. Antifeeding experiments show that cocaine is a strong deterrent to leaf feeding by the blocking of the OA reuptake transporter, thus causing a general overstimulation of the octopaminergic system that has pestistatic and pesticidal effects. Moreover, it has also been discovered that the combination of a reuptake inhibitor with a phenylethanolamine such as OA has a synergistic effect on invertebrate pests.

The invention is directed to a method of controlling an invertebrate pest, comprising contacting the pest with a pest-controlling amount of an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter, as determined by radioactive octopamine reuptake inhibition assay. Reuptake is substantially inhibited when the agent is present at a concentration of from about 10^{-12} molar (M) to about 10^{-3} M, and reuptake of a phenylethanolamine, e.g., octopamine, is inhibited from about 25 to about 100 percent as compared to reuptake by a control.

The invention is directed to a method of controlling an invertebrate pest wherein the agent is a chloroethylphenylamine and has the formula



wherein:

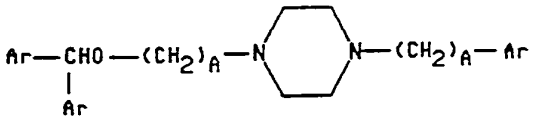
R_1 is a halogen or C_1-C_6 alkyl;

R_2 is a chlorinated C_1-C_6 alkyl;

R_3 is a C_1-C_6 alkyl; and

A is 1-6.

The invention is directed to a method of controlling an invertebrate pest wherein the agent is an aryl-1,4-dialk(en)yl piperazine and has the formula

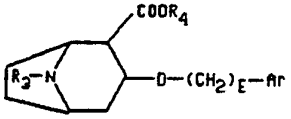


wherein:

Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl; and

A is independently 1 to 6.

The invention is also directed to a method of controlling an invertebrate pest wherein said agent is a cocaine derivative and has the formula



wherein Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl;

R_3 and R_4 are independently a C_1-C_6 alkyl;

D can be oxygen, nitrogen, sulfur, carbonyl, carboxyl, amido, C_1-C_6-N-alkyl amido, or dithio carboxyl; and

E is from 0 to 6.

The invention is also directed to a method of controlling an invertebrate pest with a compound selected from the class of tricyclic antidepressants.

The invention is also directed to a pest-controlling compound which comprises an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive octopamine reuptake inhibition assay. Reuptake is substantially inhibited

when the agent is present at a concentration of from about 10^{-12} M to about 10^{-3} M, and inhibition of reuptake of a phenylethanolamine, e.g., octopamine, is from about 25 to about 100 percent as compared to reuptake by the control.

The invention is also directed to a pesticidal composition comprising a reuptake inhibiting agent in the form of a powder, a water dispersion, an emulsion, or a dispersion, formulated together with a pesticidally inert carrier.

The invention is also directed to a synergistic pest-controlling composition which comprises: a pest-controlling amount of an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive reuptake inhibition assay; and a pest-controlling amount of a phenylethanolamine. The phenylethanolamine may be octopamine.

The invention is also directed to a process for inhibiting the feeding of an invertebrate pest comprising contacting said pest with a pest-controlling amount of an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive octopamine reuptake inhibition assay, with the proviso that said agent is not cocaine.

The invention is also directed to a process for delaying the maturation of a juvenile invertebrate by contacting it with a pest-controlling amount of an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive octopamine reuptake inhibition assay, with the proviso that said agent is not cocaine.

The phenylethanolamine reuptake inhibitors of the invention are highly selective pest control agents since vertebrate species-as opposed to invertebrate, e.g., insect, species-lack phenylethanolamine reuptake transporters selective for octopamine. In addition, these

phenylethanolamine reuptake inhibitors unexpectedly have synergistic activity when combined with other phenyethanolamines, particularly the phenylethanolamine octopamine, as an antifeeding composition.

Brief Description of the Drawings

Figure 1 is a hypothetical schematic depiction of the components involved in octopaminergic neurotransmission in invertebrates. The OA-containing neuron is shown containing OA-containing vesicles (black circles). The post-synaptic membrane is shown with an OA receptor. Release of OA into the synaptic gap allows binding of OA to the OA receptor. The pre-synaptic membrane is depicted containing OA reuptake transporter proteins. It is theorized that OA transporter blockers block the reuptake of octopamine into the pre-synaptic membrane, thereby causing increased binding of OA to susceptible OA receptors.

Figure 2 is a graph representing the antifeeding effect of the application of cocaine analogs and local anesthetics, as a reuptake inhibitor versus applied concentration. Data for cocaine, WIN 35,428, ecgonine hydrochloride, ecgonidine methyl ester mesylate, procaine, and lidocaine are shown. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At high (approximately 1%) concentrations, the % leaf remaining was approximately 90 and 95% for WIN 35,428 and cocaine, respectively, demonstrating a marked antifeeding effect for the cocaine-type class of compounds. Of the local anesthetics only lidocaine showed any activity (15% inhibition), demonstrating that local anesthetics are not necessarily indicated for antifeeding activity.

Figure 3 is a graph demonstrating the antifeeding effect of the application of chloroethyphenyl amine reuptake inhibitors versus applied concentration. Data for Xylamine and DSP-4 are shown. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At high (approximately 1-3%)

concentrations, the % leaf remaining approached 100%, demonstrating a marked antifeeding effect for this class of compounds.

Figure 4 is a graph demonstrating the antifeeding effect of the application of GBR-type reuptake inhibitors versus applied concentration. Data for GBR 12909 are shown. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At high (approximately 1%) concentrations, the % leaf remaining approached 95%, demonstrating a marked antifeeding effect for this class of compounds.

Figure 5 is a graph demonstrating the antifeeding effect of the application of tricyclic antidepressant-type reuptake inhibitors versus applied concentration. Data for desmethylipramine (DMI) and amitriptyline (AMT) are shown. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At high (approximately 3%) concentrations, the % leaf remaining approached 80 and 100%, respectively, demonstrating a marked antifeeding effect for this class of compounds.

Figure 6 is a graph demonstrating the antifeeding effect of the application of fluoxetine, an atypical antidepressant, as a reuptake inhibitor versus applied concentration. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At high (approximately 1%) concentrations, the % leaf remaining approached 85%, demonstrating a marked antifeeding effect for this class of compounds.

Figure 7 is a graph representing the antifeeding effect of the application of cocaine and OA, individually and in combination, as % leaf remaining versus time. Data for 0.6% cocaine alone, 1% OA alone, both, and a control, are shown. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At 90 hours, the % leaf remaining was approximately 5% and 35% for 1% OA and 0.6% cocaine, respectively, while the combination

shows 90% leaf remaining. This demonstrates a marked synergistic antifeeding effect for the combination over the individual components.

Figure 8 is a photograph of four tomato leaves after application of OA and cocaine as described under Figure 7. It shows that a concentration of OA which by itself was only partially effective in protecting leaves, when added to a partially effective concentration of cocaine, resulted in complete leaf protection.

Figure 9 is a graph representing the antifeeding effect of the application of OA and the known octopamine receptor blocker cyproheptadine, (see J.A. Nathanson, *Trace Amines and the Brain; Eds. Marcel Dekker*, pp. 161-190 (1976); Nathanson, J.A., *et al.*, *Proc. Natl. Acad. Sci. USA 71*:7797-7801 (1974)) individually and in combination, as % leaf remaining versus time. Data for 1% octopamine alone, 1% cyproheptadine alone, both, and a control, are shown. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At 108 hours, the % leaf remaining was approximately 10% and 55% for 1% OA alone and 1% cyproheptadine alone, respectively, while the combination shows greater than 95% leaf remaining. This demonstrates a marked synergistic antifeeding effect for the combination over the individual compounds. Fig. 9 shows that cyproheptadine alone inhibited feeding of *M. sexta* and potentiated the antifeeding effects of OA.

Figure 10 is a graph of the radiance emitted from OA-stimulated Firefly lanterns plotted versus octopamine concentration. Shown are data for OA alone and OA applied with cocaine. Fig. 10 shows that injection of OA alone into isolated firefly tails results in a dose-dependent increase in light emission. Simultaneous injection of a fixed dose of cocaine potentiates this action of OA, causing a leftward shift in the OA dose-response curve by a factor of about 10. The Firefly Lantern Assay is described in the specification under "*In vivo* Methods."

Figure 11 is a graph of the effect of cocaine on the viability of mosquito larvae when a solution of cocaine is applied to them. Percent adults surviving is graphed against cocaine concentration. At concentrations of as little as 0.01%, the percent larvae surviving to mature adulthood is approximately 50%. At 0.1%, no adults were produced.

Figure 12 is a graph of the effect of varying cocaine concentrations on the viability of *M. sexta* eggs. Percent dead ova are plotted against concentrations of cocaine ranging from 0 to 3%. At 3%, percent dead ova is greater than 95%. For the assay methods, see "*In vivo* Methods, Method 1".

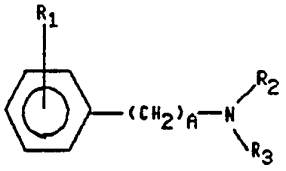
Detailed Description of the Invention

The present invention relates to the discovery of the pesticidal potential of an invertebrate-specific membrane transporter and compounds for the inhibition of the transporter. In particular, the invention relates to the phenylethanolamine reuptake transporter protein and compounds having binding specificity for it, and pesticidal/pestistatic compositions thereof.

Figure 1 is a hypothetical schematic depiction of the components involved in octopaminergic neurotransmission in invertebrates. The OA-containing neuron is shown containing OA-containing vesicles (black circles). The post-synaptic membrane is shown with an OA receptor. Release of OA into the synaptic gap allows binding of OA to the OA receptor. The pre-synaptic membrane is depicted containing OA reuptake transporter proteins. It is theorized that OA transporter blockers block the reuptake of octopamine into the pre-synaptic membrane, thereby causing increased binding of OA to susceptible OA receptors.

By the term "phenylethanolamine reuptake inhibitor" is intended compounds which block the reuptake of a phenylethanolamine compound

by a phenylethanolamine reuptake protein. A first class of such inhibitors is the chloroethylamines, represented by the formula



wherein:

R_1 is a halogen or C_1-C_6 alkyl;

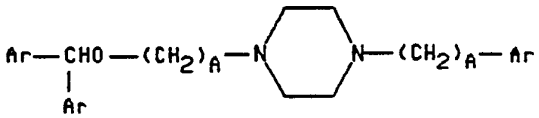
R_2 is a chlorinated C_1-C_6 alkyl;

R_3 is a C_1-C_6 alkyl; and

A is 1-6.

Synthesis of two representative chloroethylamines, xylamine and DSP-4, are reported in Fisher *et al., J. Pharmacol Exp. Ther. 226:*650 (1983); and for DSP-4 Etcheverry *et al., Brain Res. 188:*513 (1980).

A second class of phenylethanolamine reuptake inhibitors is the aryl-1,4-dialk(en)yl piperazines which have the formula



wherein:

Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6

heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl; and

A is 1-6.

By "aryl" is meant substituents such as phenyl, naphthyl, acenaphthyl, phenanthryl, etc.

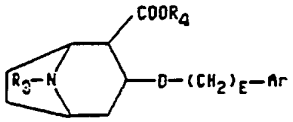
By "heteroaryl" is meant substituents such as thienyl, benzo[b]thienyl, pyrrolyl, imidazolyl, pyridyl, pyrazinyl, pyrimidinyl, 3H-indolyl, furyl, chromenyl, etc.

By "C_1-C_6 alkyl" is meant substituents such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, t-pentyl, hexyl, isohexyl, t-hexyl.

By "halogen" is meant substituents such as fluoro, chloro, bromo, and iodo.

Synthesis of GBR 12909 and many other related compounds is reported by Van der Zee, *et al., Eur. J. Med. Chem. 15*:363-370 (1980).

A third class of phenyethanolamine reuptake inhibitors is the alkaloid cocaine and related derivatives which have the formula



wherein Ar, R_3 is as defined above;

R_4 is a C_1-C_6 alkyl;

D can be oxygen, nitrogen, sulfur, carbonyl, carboxyl, amido, C_1-C_6-N-alkyl amido, or dithio carboxyl; and

E is 0 to 6.

Cocaine is a natural alkaloid derived from the leaves of the *Erythroxylon* species of coca plants. The extraction procedure is reported in Squibb, *Pharm. J. [3]* 15, 775, 796; 16, 67 (1885). Synthesis of cocaine

is reported in Willstätter *et al.*, *Ann. 434*:111 (1923). Derivatives of cocaine include, but are not limited to, cocaethylene (ecgonine ethyl ester benzoate), ecgonine hydrochloride ((-)-β-hydroxy-1-α-H,5-α-H-tropane-2-β-carboxylic acid hydrochloride) and ecgonidine methyl ester mesylate ((1R)-8-methyl-8-azabicyclo[3,2,1]oct-2-ene-2-carboxylic acid methyl ester mesylate). Synthesis of cocaethylene is reported in Merck, *Ber. 18*, 2952 (1885); Einhorn, *ibid.* 21, 47 (1888). Synthesis of ecgonine hydrochloride may be obtained via the hydrolysis of cocaine (Willstätter *et al.*, *Ann. 434*:111 (1923); Bell, Archer, *J. Am. Chem. Soc. 82*:4642 (1960)). The synthesis of ecgonidine methyl ester mesylate is reported by Martin *et al.*, *J. Ana. Toxicol. 13*(2):158-162 (1989).

The fourth class of phenylethanolamine re-uptake inhibitors include, but are not limited to, the tricyclic antidepressants exemplified by desipramine, imipramine, amoxapine, nortriptyline, protriptyline, maprotiline, doxepin, and pharmaceutically acceptable salts thereof. Methods of preparation of desipramine hydrochloride are described in Belgian Patent No. 614,616 (C.A. 58:11338C (1963)). Preparation of the free base anhydrochloride is disclosed in British Patent No. 908,788 (1962). The preparation of amoxapine, a known anti-depressant, is reported by Schmultz, J., *et al.*, *Helv. Chim. Acta 15*:245 (1967). The preparation of nortriptyline, another known anti-depressant, is reported by Hoffsommer *et al.*, *J. Org. Chem. 27*:4134 (1962). A comprehensive description of nortriptyline is provided by Hale, J.L., in *Analytical Profiles of Drug Substances*, Vol. 1, K. Florey, Ed. (Academic Press, New York, 1972), pp. 233-247. The synthesis of the anti-depressant protriptyline is described in U.S. Patent Nos. 3,244,748 and 3,271,451, and in Belgian Patent No. 617,967. Preparation of the anti-depressant maprotiline is reported in Swiss Patent Nos. 467,237 and 467,747, and in Wilhelm *et al.*, *Helv. Chim. Acta 52*:1385 (1969). Preparation of imipramine hydrochloride is reported in U.S. Patent No. 2,554,736 and in *Remington's Pharmaceutical Sciences*, Osol, R., Ed., Mack Publishing Co., Easton, PA,

p. 1040 (1980). The preparation of doxepin is reported in Stach *et al.*, *Monatsc. 93*:896 (1962), and Bickelhaupt *et al.*, *ibid. 95*:485 (1964), and in U.S. Patent No. 3,438,981 (1969). The preparation of trimipramine is reported by Jacob Messer, *Compt. Rend. 252*:2117 (1961).

The preparation of the atypical antidepressant fluoxetine, which also has demonstrated activity as a phenylethanolamine reuptake transporter inhibitor, is reported by Malloy, B.B., Schmiegel, K.K., German Patent No. 2,500,110; Eidem, U.S. Patent No. 4,314,081.

The terms "pest controlling amount" or "controlling an invertebrate pest," used throughout the specification and claims, are meant to include any pesticidal (killing) or pestistatic (preventing the host plant from being eaten, or inhibiting, maiming or generally interfering) activities of a composition against a given pest at any stage in its life cycle. Thus, these terms not only include killing, but also include the production of behavioral abnormalities (e.g., tremor, incoordination, hyperactivity, anorexia, leaf walk-off behavior) which interfere with activities such as, but not limited to, eating, molting, hatching, mobility or plant attachment. The terms also include chemosterilant activity which produces sterility in insects by preventing the production of ova or sperm, by causing death of sperm or ova, or by producing severe injury to the genetic material of sperm or ova, so that the larvae that are produced do not develop into mature progeny.

The terms also include repellant activity that protect animals, plants or products from insect attack by making food or living conditions unattractive or offensive. These repellant activities may be the result of repellants which may be poisonous, mildly toxic, or non-poisonous.

The term "substantial inhibitory activity" describes agents identified through the radioactive octopamine reuptake inhibition assay, described below. Essentially any chemical agent, present at a concentration of from about 10^{-12} molar (M) to about 10^{-3} M and demonstrating inhibition of

from about 25 to about 100 percent as compared to the control, is considered a pest-controlling agent having a substantial inhibitory activity.

The term "radioactive octopamine reuptake inhibition assay" is meant to indicate the assay described in the *In vitro* assay section. This assay is used to determine whether a given compound has any phenylethanolamine reuptake transporter-inhibiting activity. A compound that comes within this definition is one that decreases the uptake of radioactive octopamine when, over a range of concentrations from about 10^{-12} to about 10^{-3} moles per liter (M), there is a decrease of from about 25 to about 100 per cent, relative to the control.

The pest controlling agents of the present invention can be formulated as dusts, water dispersions, emulsions, and solutions. They may comprise accessory agents such as dust carriers, solvents, emulsifiers, wetting and dispersing agents, stickers, deodorants and masking agents (see, for example, *Encyclopedia of Chemical Technology*, Vol. 13, page 416 *et seq.*).

Dusts generally will contain low concentration, 0.1-20%, of the compounds, although ground preparations may be used and diluted. Carriers commonly include sulfur, silicon oxides, lime, gypsum, talc, pyrophyllite, bentonites, kaolins, attapulgite, and volcanic ash. Selection of the carrier can be made on the basis of compatibility with the desired pest control composition (including pH, moisture content, and stability), particle size, abrasiveness, absorbability, density, wettability, and cost. The agent of the invention alone or in combination and diluent is made by a variety of simple operations such as milling, solvent impregnations, fusing and grinding. Particle sizes usually range from 0.5-4.0 microns in diameter.

Wettable powders can be prepared by blending the agents of the invention in high concentrations, usually from 15-95%, with a dust carrier such as bentonite which wets and suspends properly in water. 1 to 2% of

a surface-active agent is usually added to improve the wetting and suspendibility of the powder.

The pest-controlling agents can also be used in granules, which are pelleted mixtures of the agents, usually at 2.5-10%, and a dust carrier, e.g., adsorptive clay, bentonite or diatomaceous earth, and commonly within particle sizes of 250 to 590 microns. Granules can be prepared by impregnations of the carrier with a solution or slurry of the agents and can be used principally for mosquito larvae treatment or soil applications.

The agents can also be applied in the form of an emulsion, which comprises a solution of the agents in water immiscible organic solvents, commonly at 15-50%, with a few percent of surface active agent to promote emulsification, wetting, and spreading. The choice of solvent is predicated upon solubility, safety to plants and animals, volatility, flammability, compatibility, odor and cost. The most commonly used solvents are kerosene, xylenes, and related petroleum fractions, methylisobutylketone and amyl acetate. Water emulsion sprays from such emulsive concentrates can be used for plant protection and for household insect control.

The agents can also be mixed with baits, usually comprising 1-5% of agents with a carrier especially attractive to insects. Carriers include sugar for house flies, protein hydrolysate for fruit flies, bran for grasshoppers, and honey, chocolate or peanut butter for ants.

The agents can be included in slow release formulations which incorporate non-persistent compounds, insect growth regulators and sex pheromones in a variety of granular microencapsulated and hollow fiber preparations.

The pest controlling agents of the present invention may be applied depending on the properties of the particular pest controlling compound, the habits of the pest to be controlled and the site of the application to be made. It can be applied by spraying, dusting or fumigation.

Doses of the weight of the ingredients may typically vary between 0.001 - 100 lbs/acre, preferably between 0.001 - 5 lbs/acre.

Sprays are the most common means of application and generally will involve the use of water as the principal carrier, although volatile oils can also be used. The pest-control agents of the invention can be used in dilute sprays (e.g., 0.001-10%) or in concentrate sprays in which the composition is contained at 10-98%, and the amount of carrier to be applied is quite reduced. The use of concentrate and ultra low volume sprays will allow the use of atomizing nozzles producing droplets of 30 to 80 microns in diameter. Spraying can be carried out by airplane or helicopter.

Aerosols can also be used to apply the pest controlling agents. These are particularly preferred as space sprays for application to enclosures, particularly against flying insects. Aerosols are applied by atomizing amounts of a liquified gas dispersion or bomb but can be generated on a larger scale by rotary atomizers or twin fluid atomizers.

A simple means of pest control agent dispersal is by dusting. The pest controlling agent is applied by introducing a finely divided carrier with particles typically of 0.5-3 microns in diameter into a moving air stream.

Any octopamine reuptake transporter-containing pest is treatable by the formulation of the present invention. These pests include all invertebrate pests, including, but not limited to, round worms (e.g., hookworm, trichina, ascaris); flatworms (e.g., liver flukes and tapeworms); jointed worms (e.g., leeches); mollusks (e.g., parasitic snails); and arthropods (insects, spiders, centipedes, millipedes, crustaceans (e.g., barnacles)). In particular, included among the arthropods are ticks; mites (both plant and animal); lepidoptera (butterflies and moths and their larvae); hemiptera (bugs); homoptera (aphids, scales); and coleoptera (beetles). Also included are spiders; anoplura (lice); diptera (flies and mosquitoes); trichoptera; orthoptera (e.g., roaches); odonta; thysanura

(e.g., silverfish); collembola (e.g., fleas); dermaptera (earwigs); isoptera (termites); ephemerids (mayflies); plecoptera; mallophaga (biting lice); thysanoptera; and siphonaptera (fleas); dictyoptera (roaches); psocoptera (e.g., booklice); and certain hymenoptera (e.g., those whose larva feed on leaves).

Having now generally described this invention, the same will become better understood by reference to certain specific examples which are included herein for purposes of illustration only and are not intended to be limiting unless otherwise specified. All patents and publications cited herein are fully incorporated by reference herein in their entirety.

EXAMPLES

In Vitro Assay For Determination Of Transporter Inhibitory Activity Of Compounds Of Interest

The question of whether a given compound is an inhibitor of octopamine neuronal transport can be readily determined by measuring the uptake of radioactive octopamine or similar phenylethanolamine into membrane preparations derived from insect or other invertebrate nerve tissue. To prepare membrane preparations, insect ventral nerve cord and brain ganglia from specimens of *Periplaneta americana, Manduca sexta*, or other insect or invertebrate pest are removed and homogenized in a teflon-glass homogenizer in 50 volumes of 0.32 M sucrose and then centrifuged at high speed (typically 100,000 x g) for 30 minutes to obtain a membrane pellet. The pellet is suspended in insect Ringers solution containing sodium chloride at 0°C and at a concentration of approximately 10 mg tissue per ml. Optionally, the Ringers may also contain 2 mM ascorbic acid and an inhibitor of monamine oxidase, such as pargyline. 0.2 ml or similar-sized aliquots of the membrane tissue suspension are added to ^{3}H-octopamine or similar phenylethanolamine (typically 10-40 mCi/mmol) in test tubes to a final concentration of 1 micromolar of the radioactive amine. Control tubes contain no additional compounds. Other tubes contain the compound of interest to be tested

at concentrations of from 10^{-12}M to 10^{-2}M. In addition, there are "blank" control tubes in which the sodium chloride in the insect Ringers solution has been substituted with Tris buffer or choline chloride. Alternatively, blanks may utilize: (a) an excess (>1mM) of non-radioactive octopamine, or (b) an excess (>1mM) of a transporting blocking agent, or (c) during incubation (see below), be kept at 0°C.

To measure octopamine transport (or "uptake") into membranes, the tubes are incubated at 20-35°C for 10-30 minutes, and then the contents of each tube are transferred to glass fiber filters on a filtration manifold and washed quickly under low vacuum with aliquots of ice-cold insect Ringers solution. The filters are then dried and the radioactivity remaining in the filters quantitated by liquid scintillation counting. Alternatively, the membranes may be washed by two cycles of high speed centrifugation, and the radioactivity in the final pellet quantitated by liquid scintillation counting.

After washing, radioactivity counts in the blank are subtracted from the radioactivity quantitated in the other tubes. Radioactivity remaining in the washed control membranes represents baseline phenylethanolamine transport. The radioactivity in the washed membranes from the tubes containing the compound of interest is plotted relative to control transport, and the degree of inhibition relative to control uptake is noted. To determine if the compound of interest has substantial inhibitory activity toward octopamine transport, the maximum percent decrease from control seen over the range of concentrations (10^{-12}M to 10^{-3}M) tested is determined. If this value is between 25% and 100%, then the compound is an active inhibitor of transport.

In vivo Methods

Method 1-Antifeeding Assay and Ovacidal Assay

To test the pesticidal and pestistatic effects of some of the disclosed compounds, the effects on the feeding behavior of tobacco hornworms (*Manducca sexta*) were investigated. This species is one of the several types of insects particularly susceptible to octopamine type insecticides. The ease of rearing this species from eggs in the laboratory and the ability to maintain them on artificial media, made it possible to test compounds on large numbers of larvae of the same age.

For testing, single tomato leaves were placed in a closed container, with stems hydrated by means of a small, 3 ml water-filled bottle. Compounds dissolved in water or methanol were sprayed on the tomato leaves with an ultra fine atomizer. Six 3-day-old larvae were placed on each leaf, allowed to feed for 24-108 hours, and then the percentage of leaf remaining was determined by planimetry, weight, or "blind" visual observation. An active compound was one which resulted in an increase in percentage of leaf remaining, compared with control.

Test agents were tested for ovicidal activity by dipping groups of 10-50 Manducca eggs in drug solutions for 60 seconds and then determining the percentage of eggs which produced viable larvae. Alternatively, the eggs may be sprayed with the test agent and then placed on artificial media to hatch. A compound with active ovicidal activity was one which decreased the percentage of eggs hatched, relative to control.

Method 2-Firely Lantern Assay

To measure the effects of drugs on firefly light emission, an isolated tail (terminal 3 abdominal segments containing the light organ) of a fresh adult *P. pyralis* male was mounted on a 30-g stainless steel needle and placed at the focal point of an optical system connected to a photometer-photmultiplier-chart recorder combination (see Nathanson, J.A., Characterization of octopamine-sensitive adenylate cyclase: Development of a potent and selective class of octopamine-2 receptor

agonists with toxic effects in insects, *Proc. Natl. Acad. Sci. U.S.A.* 82:599-603 (1985)). Drug (dissolved in insect saline, see Carlson, A.D., Effect of adrenergic drugs on the lantern of the larvae of the *Photinus* firefly, *J. Exp. Biol.* 48: 381-387 (1968)) was injected (3-5 μl) into the abdominal cavity dorsal to the lantern and light emission was recorded for 45 minutes or until it peaked, following which the next (larger) dose of drug was injected. In the case of animals injected with drugs other than octopamine, following the last dose, 10 mmol of octopamine (a maximally effective dose) was injected. Light production is expressd as radiance, each unit approximately equal to 1.6 nanowatts, as measured in a solid angle of 0.033 steradiants. Nathanson, J.A., Phenyliminoimidazolidines: Characterization of a Class of Potent Agonists of Octopamine-sensitive Adenylate Cyclase and Their Use in Understanding the Pharmacology of Octopamine Receptors, *Molec. Pharmacol.* 28:254-268 (1985).

Method 3-Antilarval Activity of Cocaine

Five to ten 2-5-day old mosquito larvae, reared from eggs, were placed in small bottles containing 3 ml of water, 2 ml of air space, and a perforated cap open to the air. The compound of interest was dissolved in water or appropriate solvent and added to make a final concentration of from 0.00001 to 1%. The bottles were capped and observed at daily intervals for the number of living larvae, pupae, and emerging adults. After two weeks, the final number of adults emerging as a percentage, relative to control, is plotted as a function of drug concentration.

Example 1

Feeding Inhibition of *M. sexta* on cocaine-sprayed tomato leaves

First instar *M. sexta* larvae were placed upon tomato leaves presprayed with various concentrations of cocaine. After a few minutes of exposure to cocaine-sprayed leaves, larvae displayed marked behavioral abnormalities, including rearing, tremors, and walk-off activity. These

behaviors increased in intensity as larvae began to feed and, at higher concentrations of cocaine, animals died after 48-72 hours. As a result, leaves were protected, and Fig. 2 shows the dose-response relationship on inhibition of feeding that was observed. Cocaine and the cocaine derivative WIN 35,428 (CFT Naphthalene disulfonate((-)-2-beta-carbomethoxy-3-beta-(4-fluorophenyl) tropane-1,5-naphthalene disulfonate) (Research Biochemicals Inc., Natick, MA) (Madras, B., *et al.*, *Mol. Pharmacol. 36*:518 (1989)) show a marked inhibition on leaf feeding. At 1% spray concentration, cocaine and WIN 35,428 show 90 and 95% leaf remaining, respectively. Ecgonine hydrochloride ((-)-beta-hydroxy-1-alpha-H, 5-alpha-H-tropane-2-beta-carboxlic acid hydrochloride) and Ecgonidine methyl ester mesylate ((1R)-8-methyl-8-azabicyclo[3,2,1]oct-2-ene-2-carboxylic acid methyl ester mesylate) displayed no antifeeding effect, suggesting that both the tropane ring and the benzoyl substituent are necessary for activity. Control experiments using procaine and lidocaine showed that leaf protection was not due to local anesthetic effects, an observation consistent, also, with the fact that larvae exposed to cocaine initially demonstrated hyperactivity rather than hypoactivity. As will be described in the examples which follow, the pest-controlling mechanism of action of cocaine and the other reuptake blockers is through their action on blocking the reuptake of OA (rather than some other amine).

Example 2

Feeding Inhibition of Known Amine Reuptake Blockers

Pharmacological evidence from vertebrates supports the presence of distinct reuptake sites (amine transporter proteins) for DA, NE, and serotonin (5-HT), as discussed *infra*. The potential insect antifeeding effects of several other known amine reuptake blockers, representing three more classes of compounds with varying degrees of selectivity toward DA, NE, and 5-HT, were examined for their possible insecticidal

activity. Six compounds had antifeeding effects, including desmethylimipramine (DMI) (a better NE than 5-HT uptake blocker), amitriptyline (AMT) (a better 5-HT than NE reuptake blocker), xylamine (XYL) and DSP-4 (both primarily NE uptake blockers), fluoxetine (FLU) (primarily a 5-HT reuptake blocker), and GBR 12909 (a relatively selective DA reuptake blocker). Fig. 3 shows the two chloroethylphenylamine compounds Xylamine and DSP-4. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At high (approximately 1-3%) concentrations, the % leaf remaining approached 100%, demonstrating a marked antifeeding effect for the chloroethylphenylamine class of compounds. Fig. 4 shows the effect of the aryl-1,4-dialk(en)yl piperazine compound GBR 12909 (1-(2-[bis-(4-fluorophenyl)methoxy]ethyl)-4-(3-phenyl-2-propenyl)piperazine) (Research Biochemicals, Inc., Natick, MA) are shown. At high (approximately 1%) concentrations, the % leaf remaining approached 95%, demonstrating a marked antifeeding effect for this class of compounds. Fig. 5 shows the antifeeding effect of two representative tricyclic antidepressants, desmethylimipramine (DMI) and amitriptyline (AMT) (Sigma Chemical, St. Louis, MO). At high (approximately 3%) concentrations, the % leaf remaining approached 80 and 100%, respectively. Fig. 6 depicts the leaf antifeeding effect of the atypical antidepressant fluoxetine. At high (approximately 1%) concentrations, the % leaf remaining approached 85%, demonstrating a marked antifeeding effect for this class of compounds. In other experiments (not shown), the NE and DA uptake blocker, mazindol, exerted no antifeeding activity.

When the rank order of these various compounds for insect antifeeding potency is compared with their relative potency at blocking DA reuptake into rat brain synaptosomes (Table 1) (Andersen, P., *J. Neurochem.* *48*:1887-96 (1987); Bonnet, J., *et al.*, *Eur. J. Pharmacol.*

126:211-22 (1986); Berger, P., *et al.*, *Eur. J. Pharmacol. 107*:289-90 (1985)), it is clear that the pharmacology differs in the two instances.

Table 1

RANK ORDER EFFECT ON BLOCKING DA UPTAKE
GBR >> MAZ > COC >> DMI = AMT = FLUOX

RANK ORDER ANTIFEEDING EFFECT IN *MANDUCA*
GBR > COC = DMI = AMT > DSP = XYL = FLUOX >> MAZ

RANK ORDER EFFECT ON BLOCKING NE UPTAKE
MAZ > DMI >> AMT = GBR = COC

RANK ORDER EFFECT ON BLOCKING 5-HT UPTAKE
FLUOX > AMT >> DMI = COC > GBR > MAZ

Thus, either the pharmacological selectivity of the DA transporter of insects is different from that in mammals or these drugs are affecting the uptake of some other amine. Table 1 shows that the rank order potency of antifeeding effect in *Manduca* is also different from the relative selectivity of these compounds in blocking NE uptake (Richeson and Pfenning, *Eur. J. Pharmacol. 104*:277-286 (1984); Pacholczyk, T., *et al.*, *Nature 350*:350-52 (1991)) or serotonin uptake (Richeson and Pfenning, *ibid.*; Blakely, R., *et al.*, *Nature 354*:66-70 (1991)), results which suggest that the antifeeding activity observed is not due to an effect on NE or 5-HT uptake.

Of interest, the behavioral effects of these compounds in *Manduca* are similar, not only to the effects of cocaine, but also to the adverse behavioral effects that we have previously observed with OA receptor agonists. Thus, if the antifeeding effects of the reuptake blockers are not due to their action on DA, NE or 5-HT, it is likely that the compounds are working through a blockade of OA reuptake, thereby augmenting OA neurotransmission and functionally acting as OA agonists. This will be demonstrated below in Examples 3-5 (Figs. 7-8). Work done over a decade ago by Evans (Evans, P.D., *J. Neurochem. 30*:1015-1022 (1978)) showed the presence, in cockroach nerve cord, of high affinity uptake for OA which is blockable by DMI. Unfortunately (in part due to the relative

lack of selectivity of blockers then available), pharmacological characterization of this site was, at the time, insufficient to determine whether the characteristics of the OA uptake were distinct from those of mammalian amine reuptake sites.

Example 3

Synergistic Antifeeding Effect of Octopamine Administered with Cocaine

Fig. 7 shows a concentration of OA, which by itself, was only partially effective in protecting leaves, and when added to a partially effective concentration of cocaine, resulted in complete leaf protection. Data for 0.6% cocaine alone, 1% OA alone, both, and a control, are shown. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At 90 hours, the % leaf remaining was approximately 5% and 35% for 1% OA and 0.6% cocaine, respectively, while the combination shows 90% leaf remaining. This demonstrates a marked synergistic antifeeding effect for the combination over the individual components when tested seperately. Fig. 8 is a photograph of four tomato leaves after application of OA and cocaine as just described. It shows that a concentration of OA which by itself was only partially effective in protecting leaves, when added to a partially effective concentration of cocaine, resulted in complete leaf protection.

Evidence indicating a lack of involvement of DA was obtained from a similar experiment in which DA, alone (at doses up to twice that of OA), caused no antifeeding effects in *Manduca* and addition of DA to cocaine caused no further increase in antifeeding effect. Compared to OA, NE and 5-HT (when used alone) were also significantly weaker than OA as antifeeding agents.

Example 4

Antifeeding Effect of Cyproheptadine

Cyproheptadine is an antagonist of OA-activated insect adenylate cyclase (J.A. Nathanson, *Trace Amines and the Brain; Eds. Marcel Dekker*, pp. 161-190 (1976); Nathanson, J.A., *et al.*, *Proc. Natl. Acad. Sci. USA* *71*:7797-7801 (1974)). Figure 9 is a graph representing the antifeeding effect of the application of octopamine and the known octopamine receptor blocker cyproheptadine,(see J.A. Nathanson, supra; Nathanson, J.A., *et al.*, supra) individually and in combination, as % leaf remaining versus time. Data for 1% octopamine alone, 1% OA alone, both, and a control, are shown. Antifeeding inhibition was measured according to the *in vivo* assay for tomato leaves, described *infra* under "*In vivo* Methods." At 108 hours, the % leaf remaining was approximately 10% and 55% for 1% OA alone and 1% cyproheptadine alone, respectively, while the combination shows greater than 95% leaf remaining. This demonstrates a marked synergistic antifeeding effect for the combination over the individual compounds.

Fig. 9 shows, surprisingly, that cyproheptadine alone inhibited feeding of *Manduca* and that, even more perplexing, instead of blocking, potentiated the antifeeding effects of OA. This unexpected action can now be explained by the structure of cypoheptadine which is virtually identical to that of amitriptyline, an amine reuptake blocker. In fact, the literature indicates that cyproheptadine can block amine reuptake (Evans, P.D., *J. Neurochem.* *30*:1015-1022 (1978)). Thus, behaviorally, this compound's potentiating effect on reuptake is greater than its effect on OA receptor blockade, not only explaining its antifeeding activity but also providing additional support for using OA uptake as a potential target for pesticides and pestistatics.

Example 5

Potentiation of OA Stimulation in the Firefly Lantern

Additional and more direct evidence for the ability of cocaine to potentiate OA neurotransmission has come from an experiment carried out using the OA-mediated regulation of light emission in the firefly lantern. This assay is described *infra* under "*In vivo* Methods." As mentioned, this system is purely octopaminergic without any evidence of membrane receptors for DA, 5-HT or NE. Fig. 10 shows that injection of OA into isolated firefly tails results in a dose-dependent increase in light emission. Simultaneous injection of a fixed dose of cocaine potentiates this action of OA, causing a leftward shift in the OA dose-response curve by a factor of about 10.

These experiments not only support an action of cocaine on OA transmission but, because they were run with zero Ca++/high Mn++, are also consistent with the expected effect of cocaine on neurotransmitter reuptake, rather than release. (These ionic conditions prevent synaptic release (J.A. Nathanson, *Trace Amines and the Brain; Eds. Marcel Dekker*, pp. 161-190 (1976); Nathanson, J.A., *Mol. Pharmacol. 28*:254-268 (1985)).

Example 6

Effect of Cocaine on Mosquito Larvae

The result of the application of a solution of cocaine to invertebrate larvae is shown in Figure 11. The assay is described in the Methods section under "*In vivo* Methods - Method 3."

Figure 11 is a graph of the effect of cocaine on the viability of mosquito larvae when a solution of cocaine is applied to them. Percent living adults is graphed against cocaine concentration. At concentrations of as little as 0.01%, the percent larvae surviving to mature adulthood is approximately 50%. At 0.1%, no adults were produced. A strong correlation between cocaine concentration and larvacidal activity of cocaine is demonstrated.

Example 7

Ovacidal Activity of Cocaine

The result of the application of a solution of cocaine to invertebrate eggs is shown in Figure 12. For the assay methods, see "*In vivo* Methods - Method 1." Figure 12 is a graph of the effect of varying cocaine concentrations on the viability of *M. sexta* eggs. Percent dead ova are plotted against concentrations of cocaine ranging from 0 to 3%. At 3%, Percent Dead Ova is greater than 95%. A strong correlation between cocaine concentration and ovacidal activity of cocaine is demonstrated.

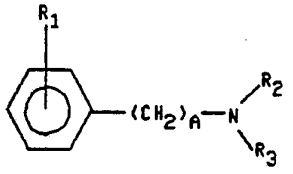
Although the foregoing invention has been described by way of illustration and example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the invention, as limited only by the scope of the appended claims.

We claim:

1. A method of controlling an invertebrate pest, comprising contacting said pest with a pest-controlling amount of an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive octopamine reuptake inhibition assay, with the proviso that said agent is not cocaine.

2. The method of claim 1 wherein said reuptake is substantially inhibited when said agent is present at a concentration of from about 10^{-12} M to about 10^{-3} M, and said inhibition is from about 25 to about 100 percent as compared to control reuptake.

3. The method of claim 1 wherein said agent is a chloroethylphenylamine and has the formula



wherein:

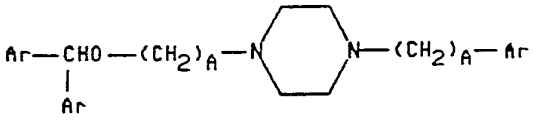
R_1 is a halogen or C_1-C_6 alkyl;

R_2 is a chlorinated C_1-C_6 alkyl;

R_3 is a C_1-C_6 alkyl; and

A is 1-6.

4. The method of claim 1 wherein said agent is an aryl-1,4-dialk(en)yl piperazine and has the formula

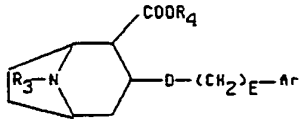


wherein:

Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl; and

A is independently 1 to 6.

5. The method of claim 1 wherein said agent is a cocaine derivative and has the formula



wherein Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6

haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl;

R_3 and R_4 are independently C_1-C_6 alkyl;

D can be oxygen, nitrogen, sulfur, carbonyl, carboxyl, amido, C_1-C_6-N-alkyl amido, or dithio carboxyl; and

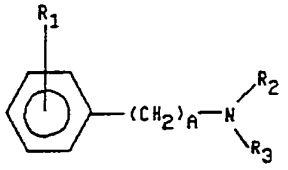
E is 0 to 6.

6. The method of claim 1 wherein said agent comprises a compound selected from the class of tricyclic antidepressants.

7. A pest-controlling composition comprising a compound which comprises an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive octopamine reuptake inhibition assay, together with a pesticidally inert carrier.

8. The pest-controlling composition of claim 7 wherein said phenylethanoloamine reuptake transporter is specific for octopamine.

9. The pest-controlling composition of claim 8 wherein said agent has the formula



wherein:

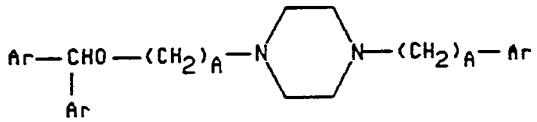
R_1 is a halogen or C_1-C_6 alkyl;

R_2 is a chlorinated C_1-C_6 alkyl;

R_3 is a C_1-C_6 alkyl; and

A is 1-6.

10. The pest-controlling composition of claim 8 wherein said agent has the formula



wherein:

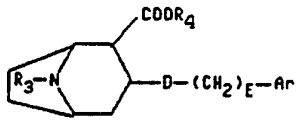
Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15}

dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl; and

A is independently 1 to 6.

11. The pest-controlling composition of claim 8 wherein said agent comprises a compound selected from the class of tricyclic antidepressants.

12. The pest-controlling composition of claim 8 wherein said agent has the formula:



wherein

Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl;

R_3 and R_4 are independently C_1-C_6 alkyl;

D can be oxygen, nitrogen, sulfur, carbonyl, carboxyl, amido, C_1-C_6-N-alkyl amido, or dithio carboxyl; and

E is 0 to 6.

13. The pest-controlling composition of claim 8 wherein reuptake is substantially inhibited when said agent is present at a concentration of from about 10^{-12} M to about 10^{-3} M, and said inhibition is from about 25 to about 100 percent as compared to control reuptake.

14. The pest-controlling composition of claim 8 in the form of a powder, a water dispersion, an emulsion, or a dispersion.

15. The pest-controlling composition of claim 8 wherein said inert carrier is selected from the group consisting of sulfur, silicon oxides, lime, gypsum, talc, pyrophyllite, bentonite, kaolins, allapulgite, and volcanic ash.

16. A synergistic pest-controlling composition which comprises:

(a) an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive reuptake inhibition assay; and

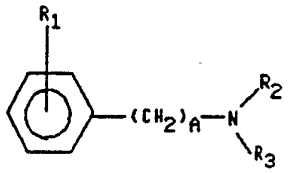
(b) a phenylethanolamine;

wherein said phenylethanolamine and said agent are present in a pest-controlling amount.

17. The pest-controlling composition of claim 16 wherein reuptake is substantially inhibited when said agent is present at a concentration of from about 10^{-12} M to about 10^{-3} M, and said inhibition is from about 25 to about 100 percent as compared to control reuptake.

18. The pest-controlling composition of claim 16 wherein said phenylethanolamine is octopamine.

19. The pest-controlling composition of claim 16 wherein said agent is a chloroethylphenylamine and has the formula



wherein:

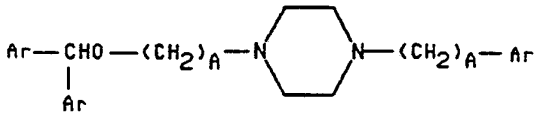
R_1 is a halogen or C_1-C_6 alkyl;

R_2 is a chlorinated C_1-C_6 alkyl;

R_3 is a C_1-C_6 alkyl; and

A is 1-6.

20. The pest-controlling composition of claim 16 wherein said agent is an aryl-1,4-dialk(en)yl piperazine and has the formula



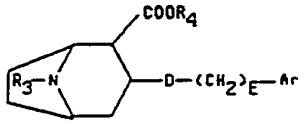
wherein:

Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkyl-

sulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl; and

A is independently 1 to 6.

21. The pest-controlling composition of claim 16 wherein said agent is a cocaine derivative and has the formula



wherein Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl;

R_3 and R_4 are independently C_1-C_6 alkyl;

D can be oxygen, nitrogen, sulfur, carbonyl, carboxyl, amido, C_1-C_6-N-alkyl amido, or dithio carboxyl; and

E is 0 to 6.

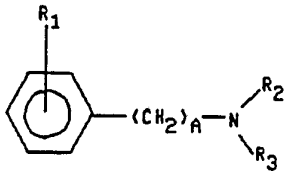
22. The pest-controlling composition of claim 16 wherein said agent comprises a compound selected from the class of tricyclic antidepressants.

23. A process for inhibiting the feeding of an invertebrate pest comprising contacting said pest with a pest-controlling amount of an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive octopamine reuptake inhibition assay, with the proviso that said agent is not cocaine.

24. The process of claim 23, wherein said agent is selected from the group consisting of xylamine, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4), 1-(2-[bis-(4-fluorophenyl)methoxy]ethyl)-4-(3-phenyl-2-propenyl)piperazine (GBR 12909), amitryptiline, desipramine, fluoxetine, and CFT Naphthalene disulfonate ((-)-2-beta-carbomethoxy-3-beta-(4-fluorophenyl) tropane-1,5-naphthalene disulfonate) (WIN 35,428).

25. The process of claim 23 wherein said reuptake is substantially inhibited when said agent is present at a concentration of from about 10^{-12} M to about 10^{-3} M, and said inhibition is from about 25 to about 100 percent as compared to control reuptake.

26. The process of claim 23 wherein said agent is a chloroethylphenylamine and has the formula



wherein:

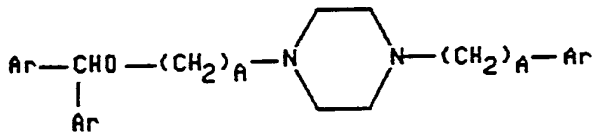
R_1 is a halogen or C_1-C_6 alkyl;

R_2 is a chlorinated C_1-C_6 alkyl;

R_3 is a C_1-C_6 alkyl; and

A is 1-6.

27. The process of claim 23 wherein said agent is an aryl-1,4-dialk(en)yl piperazine and has the formula



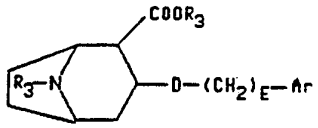
wherein:

Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl; and

A is independently 1 to 6.

28. The process of claim 23 wherein said agent is a cocaine derivative and has the formula

wherein Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo,



iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl;

R_3 and R_4 are independently a C_1-C_6 alkyl;

D can be oxygen, nitrogen, sulfur, carbonyl, carboxyl, amido, C_1-C_6-N-alkyl amido, or dithio carboxyl; and

E is 0 to 6.

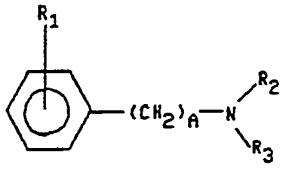
29. The process of claim 23 wherein said agent comprises a compound selected from the class of tricyclic antidepressants.

30. A process for delaying the maturation of a juvenile invertebrate by contacting it with a pest-controlling amount of an agent having substantial inhibitory activity toward a phenylethanolamine reuptake transporter as determined by radioactive octopamine reuptake inhibition assay, with the proviso that said agent is not cocaine.

31. The process of claim 30 wherein said reuptake is substantially inhibited when said agent is present at a concentration of

from about 10^{-12} M to about 10^{-3} M, and said inhibition is from about 25 to about 100 percent as compared to control reuptake.

32. The process of claim 30 wherein said agent is a chloroethylphenylamine and has the formula



wherein:

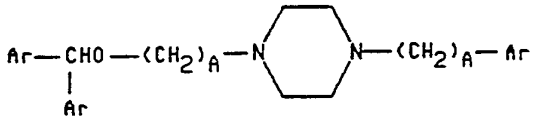
R_1 is a halogen or C_1-C_6 alkyl;

R_2 is a chlorinated C_1-C_6 alkyl;

R_3 is a C_1-C_6 alkyl; and

A is 1-6.

33. The process of claim 30 wherein said agent is an aryl-1,4-dialk(en)yl piperazine and has the formula



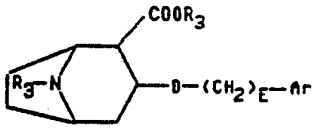
wherein:

Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl

ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl; and

A is independently 1 to 6.

34. The process of claim 30 wherein said agent is a cocaine derivative and has the formula



wherein Ar is independently aryl or heteroaryl wherein aryl or heteroaryl can be substituted by hydrogen, halogen such as chloro, fluoro, bromo, iodo, CF_3, C_1-C_6 alkoxy, C_2-C_6 dialkoxymethyl, C_1-C_6 alkyl, cyano, C_3-C_{15} dialkylaminoalkyl, carboxy, carboxamido, C_1-C_6 haloalkyl, C_1-C_6 haloalkylthio, allyl, aralkyl, C_3-C_6 cycloalkyl, aroyl, aralkoxy, C_2-C_6 acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, an aryl ring fused to a substituted benzene ring, a substituted aryl ring fused to a benzene ring, a heteroaryl ring fused to a benzene ring, a substituted heteroaryl ring fused to a benzene ring, C_3-C_6 heterocycloalkyl, a C_3-C_6 heterocycloalkyl ring fused to a benzene ring, C_1-C_6 alkylthio, C_1-C_6 alkylsulfonyl, C_1-C_6 haloalkylsulfonyl, C_1-C_6 alkylsulfinyl, C_1-C_6 haloalkylsulfinyl, arylthio, C_1-C_6 haloalkoxy, amino, C_1-C_6 alkylamino, C_2-C_{15} dialkylamino, hydroxy, carbamoyl, C_1-C_6 N-alkylcarbamoyl, C_2-C_{15} N,N-dialkylcarbamoyl, nitro and C_2-C_{15} dialkylsulfamoyl;

R_3 and R_4 are independently a C_1-C_6 alkyl;

D can be oxygen, nitrogen, sulfur, carbonyl, carboxyl, amido, C_1-C_6-N-alkyl amido, or dithio carboxyl; and

E is 0 to 6.

35. The process of claim 30 wherein said agent comprises a compound selected from the class of tricyclic antidepressants.

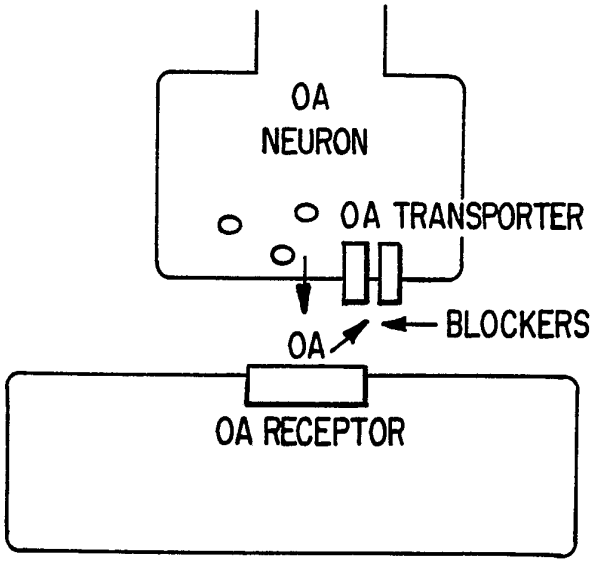

OA
NEURON
OA TRANSPORTER
OA
BLOCKERS
OA RECEPTOR

FIG. 1

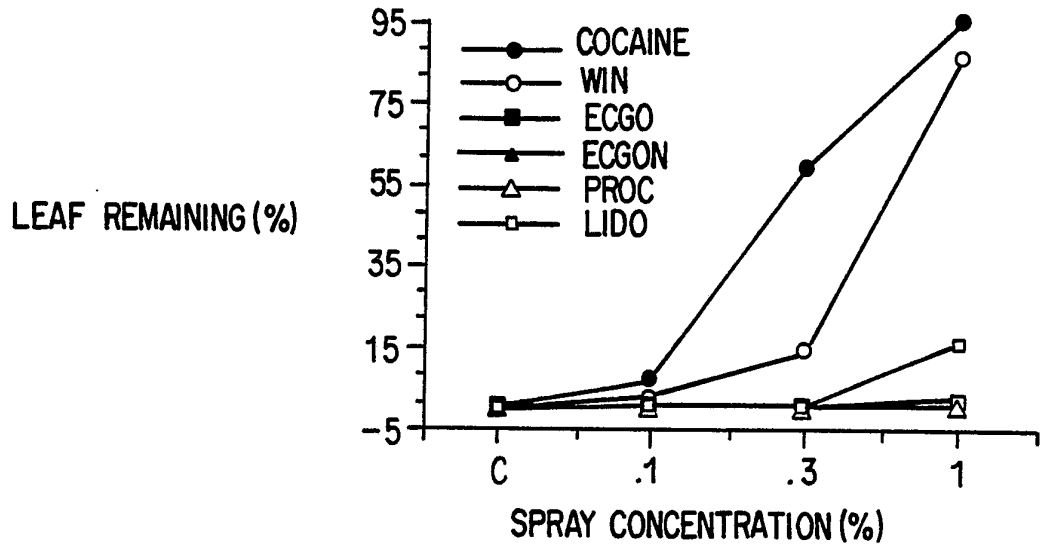

LEAF REMAINING (%)
95
75
55
35
15
-5
C
.1
.3
1
SPRAY CONCENTRATION (%)
COCAINE
WIN
ECGO
ECGON
PROC
LIDO

FIG. 2

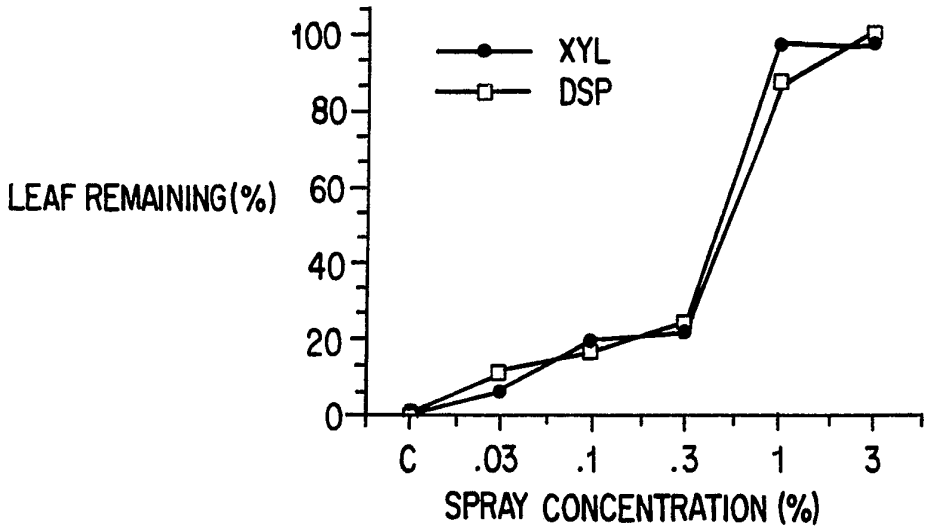

100
80
60
40
20
0
LEAF REMAINING(%)
XYL
DSP
C
.03
.1
.3
1
3
SPRAY CONCENTRATION (%)

FIG. 3

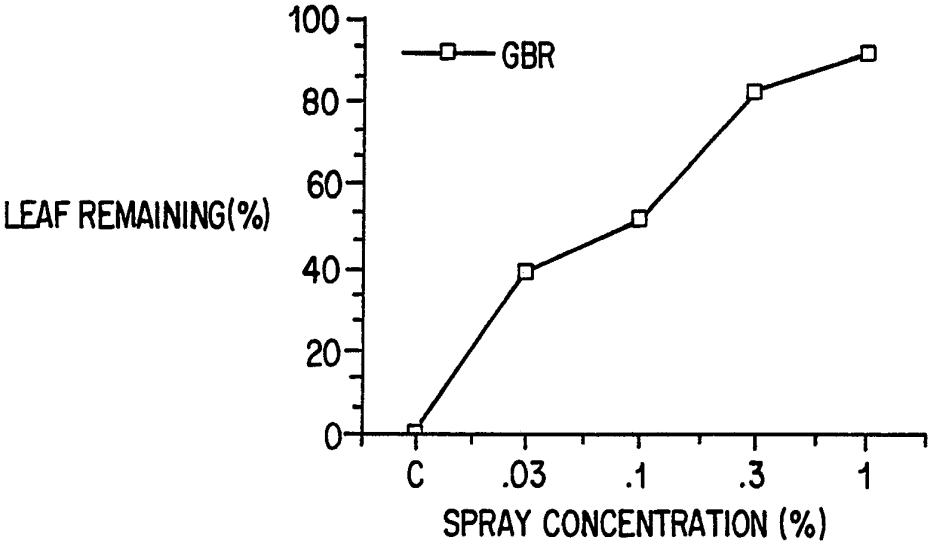

100
80
60
40
20
0
LEAF REMAINING(%)
GBR
C
.03
.1
.3
1
SPRAY CONCENTRATION (%)

FIG. 4

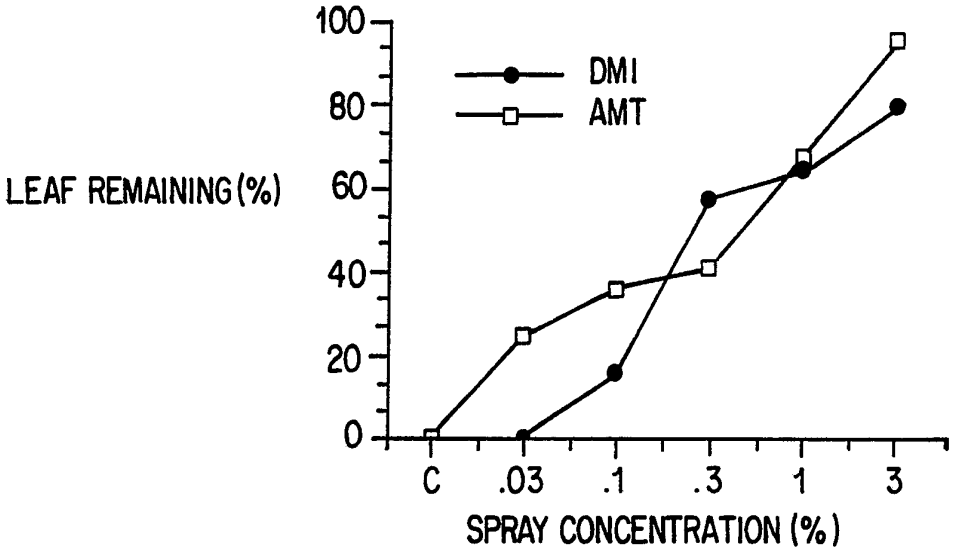

100
80
60
40
20
0
LEAF REMAINING (%)
DMI
AMT
C
.03
.1
.3
1
3
SPRAY CONCENTRATION (%)

FIG. 5

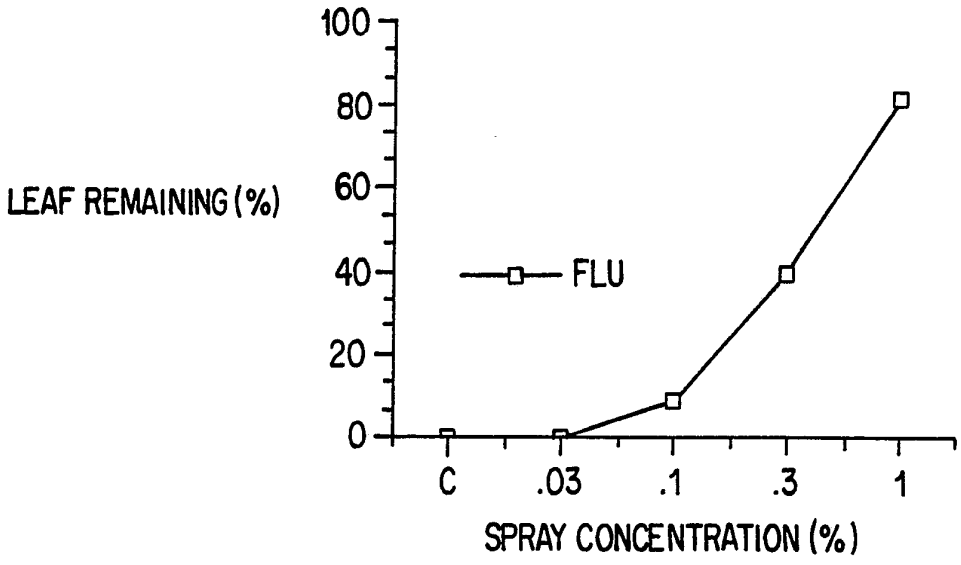

100
80
60
40
20
0
LEAF REMAINING (%)
FLU
C
.03
.1
.3
1
SPRAY CONCENTRATION (%)

FIG. 6

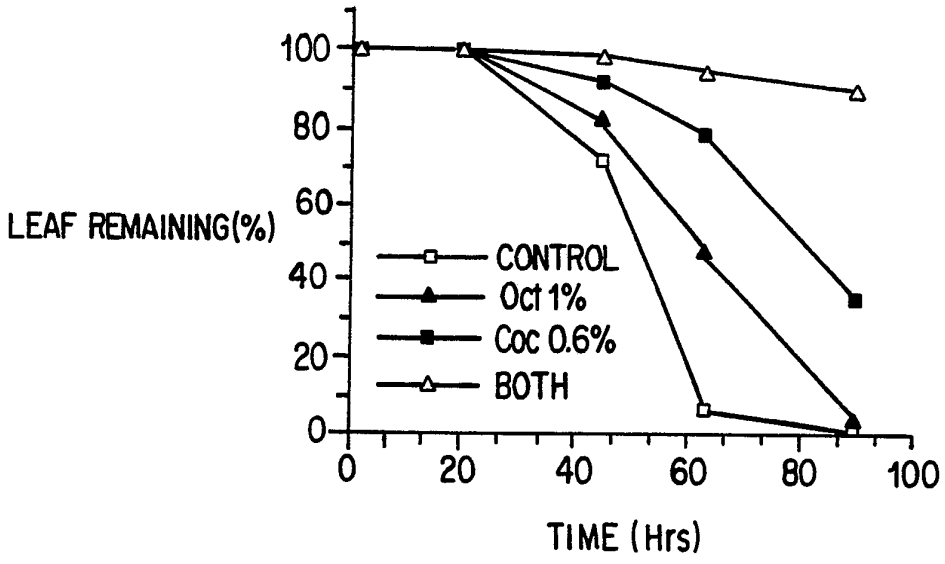

LEAF REMAINING(%)
100
80
60
40
20
0
0
20
40
60
80
100
TIME (Hrs)
CONTROL
Oct 1%
Coc 0.6%
BOTH

FIG.7

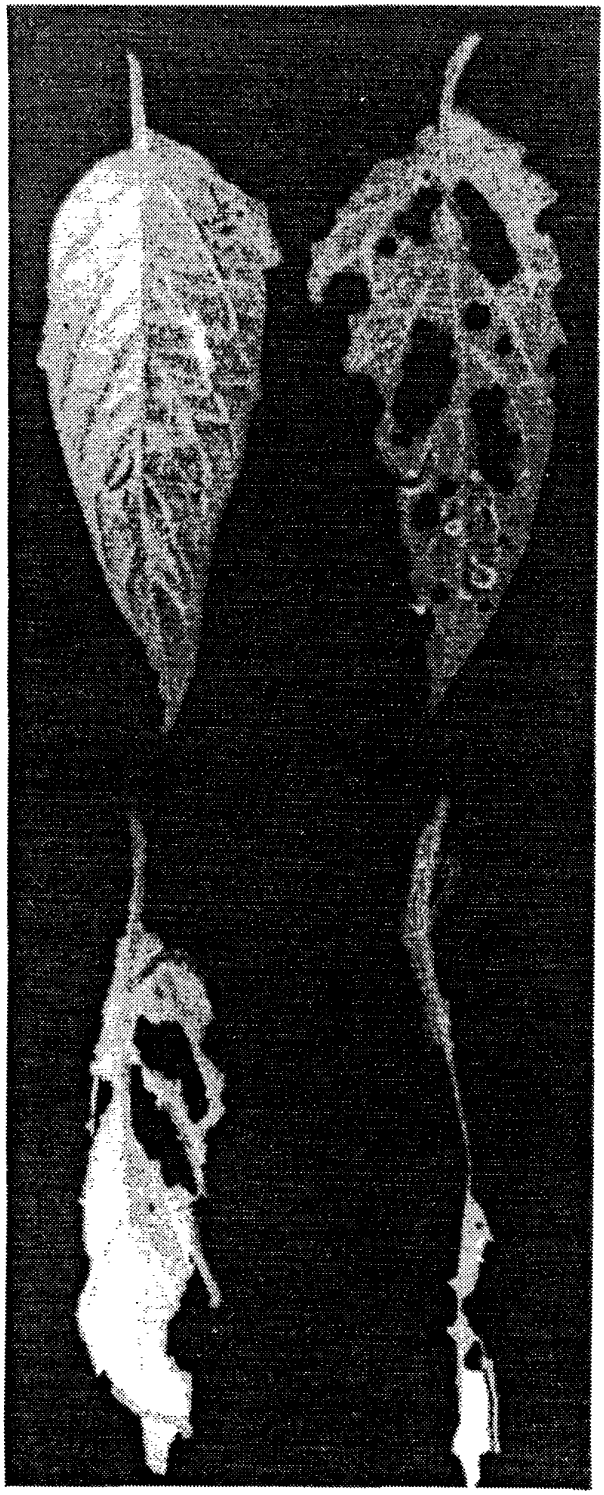


FIG. 8

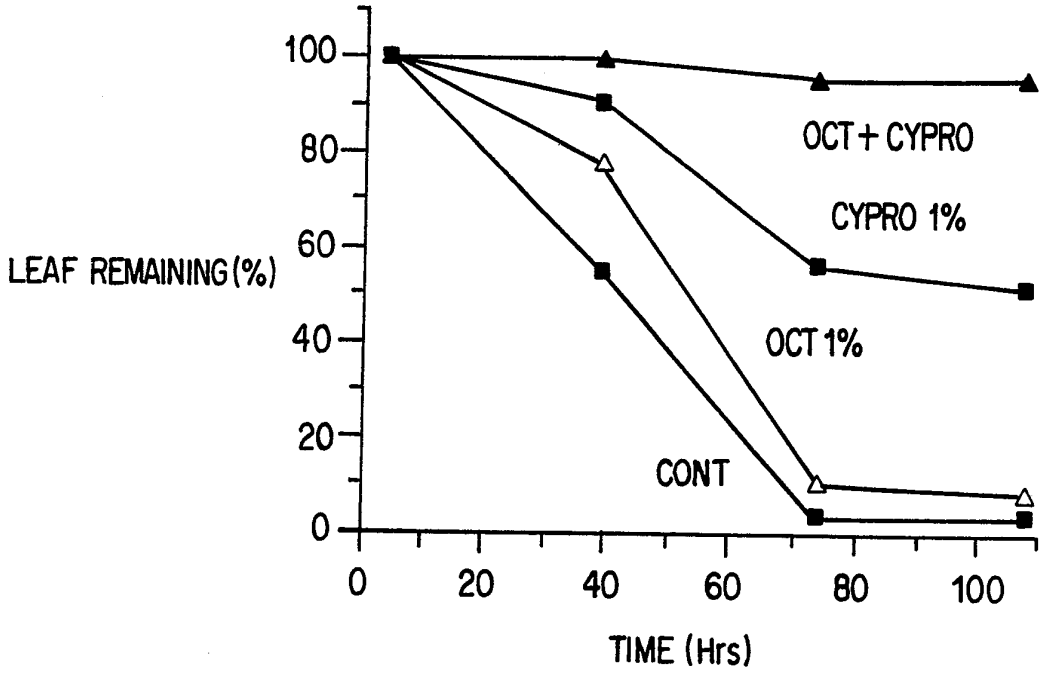

LEAF REMAINING (%)
100
80
60
40
20
0
0
20
40
60
80
100
TIME (Hrs)
OCT + CYPRO
CYPRO 1%
OCT 1%
CONT

FIG. 9

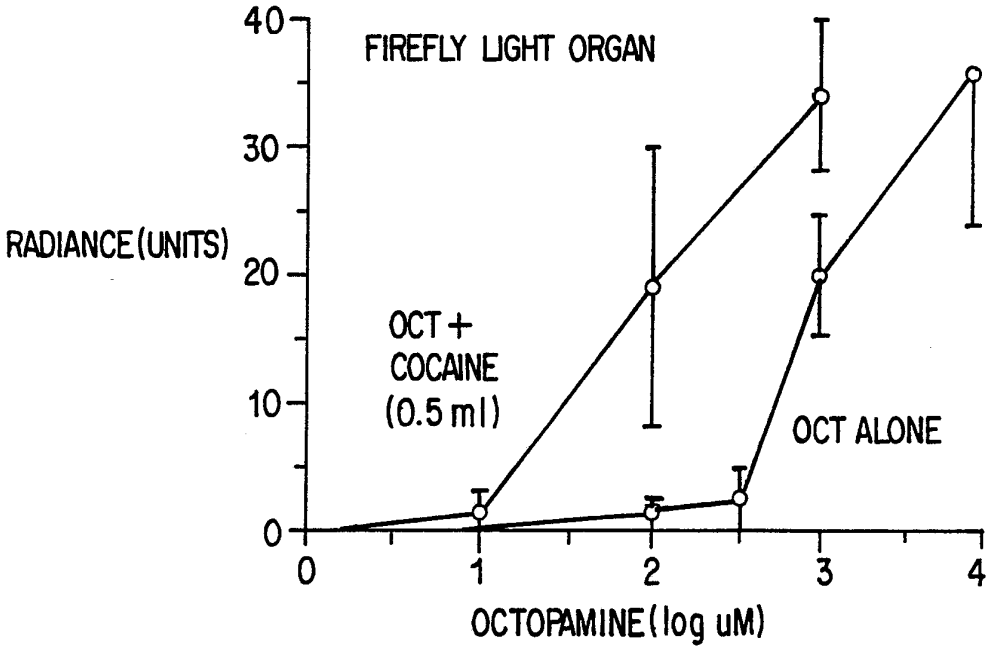

RADIANCE (UNITS)
40
30
20
10
0
0
1
2
3
4
OCTOPAMINE (log uM)
FIREFLY LIGHT ORGAN
OCT + COCAINE (0.5 ml)
OCT ALONE

FIG. 10

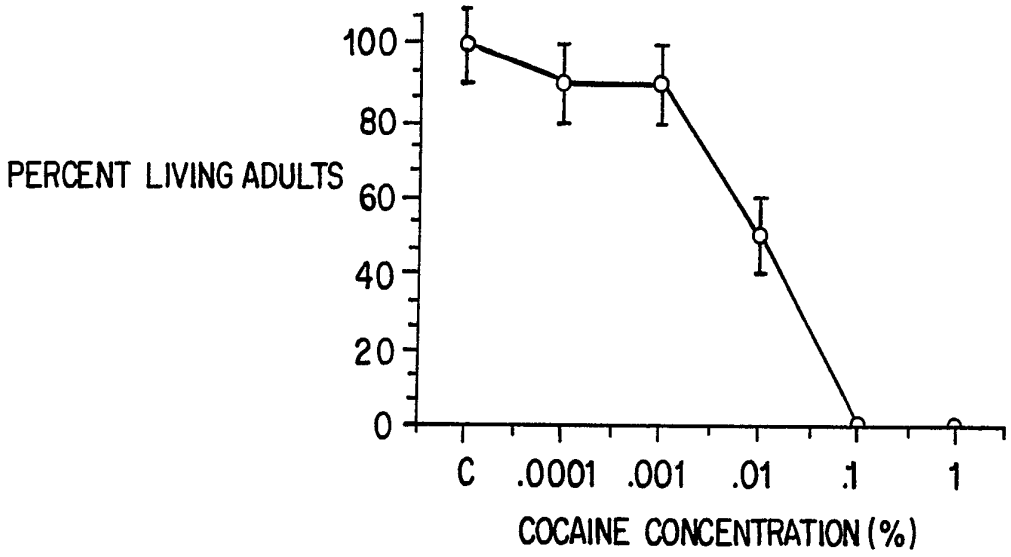

PERCENT LIVING ADULTS
100
80
60
40
20
0
C
.0001
.001
.01
.1
1
COCAINE CONCENTRATION (%)

FIG. 11

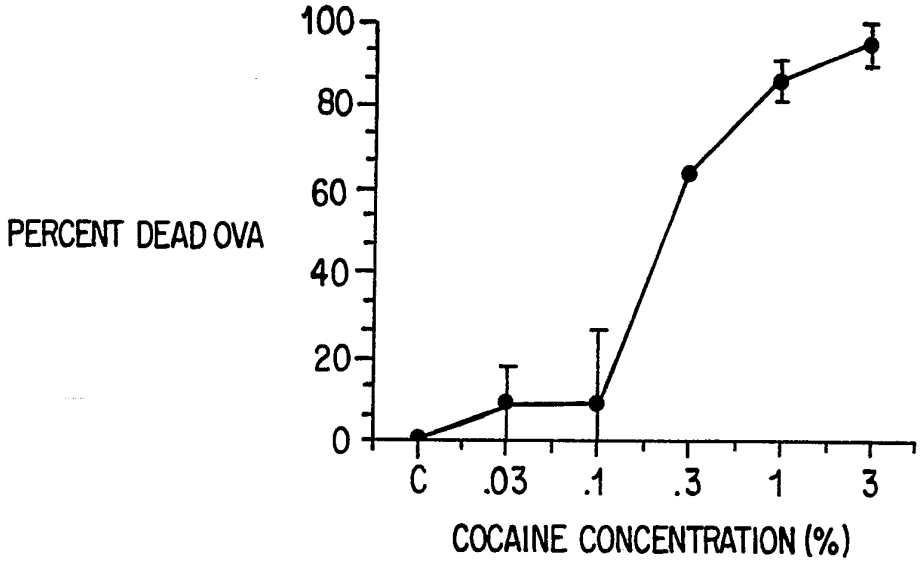

PERCENT DEAD OVA
100
80
60
40
20
0
C
.03
.1
.3
1
3
COCAINE CONCENTRATION (%)

FIG. 12

INTERNATIONAL SEARCH REPORT

Int tional application No.
PCT/US92/05473

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A01N 33/02,33/04
US CL :514/649,654,655
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 564/374,384

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)

STN ON LINE, FILES REG. & CA.
Pesticid#, insecticide#, molluscide#, parasiticide#, Roach?, Blattar?, invertebra?, Lice?, Cockroach?, fly, flies.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,678,775 (NATHANSON) 07 July 1987, See claims 1-9 and 19-20.	1-3,7-9, 13-19
Y	US, A, 4,783,457 (NATHANSON) 08 November 1988, See columns 3-5 and claims 1-7.	1-3,7-9, 13-19
Y	US, A, 4,902,690 (NATHANSON) 20 February 1990, See columns 4-6 and claims 1-14.	1-3,7-9, 13-19
Y	US, A, 4,892,871 (NATHANSON) 09 January 1990, See column 11.	1-3,7-9, 13-19
Y	US, A, 3,781,443 (FULLER ET AL.) 25 December 1973, See columns 1 and 6.	7-9,14
Y	US, A, 5,059,422 (FISHBEIN ET AL.) 22 October 1991, See claims 1-3 and 9.	7-9
Y	DE, A, 3,234,995 (HORSTMANN ET AL.) 22 September 1982. see entire document	7-9 and 14-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
17 OCTOBER 1992	05 NOV 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer JOHN PAK Telephone No. (703) 308-4538

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/05473

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATHANSON, J.A. "Phenylethanolamine Receptors as Selective Targets for Pesticide Action". in: HOLLINGWORTH, R.M. et al., eds., Sites of Action for Neurotoxic Pesticides, 1987, (Washington, DC, American Chemical Society), pp. 154-161.	1-3,7-9, 13-19
X	US, A, 4,541,954 (BOROWSKI ET AL.) 17 September 1985, See column 2, lines 10-16 and 31-32.	7-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/05473

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 3,9 and 19, which are drawn to a composition and method for controlling an invertebrate pest by utilizing a chloroethylphenylamine compound, classified in 514/646 + .

II. Claims 4,10 and 20, which are drawn to a composition and method for controlling an invertebrate pest by utilizing an aryl-1,4-dialk(en)yl piperazine compound, classified in 514/255.

III. Claims 5,12 and 21, which are drawn to a composition and method for controlling an invertebrate pest by utilizing a bicyclic cocaine derivative, classified in 514/299.

IV. Claims 6,11 and 22, which are drawn to a composition and method for controlling an invertebrate pest by utilizing a tricyclic antidepressant compound, classified in 514/656 and in other subclasses depending on the structures of the various tricyclic antidepressant compounds.

V. Claim 26, which is drawn to a method of inhibiting the feeding of an invertebrate pest by utilizing a chloroethylphenylamine compound, classified in 514/646 +.

VI. Claim 27, which is drawn to a method of inhibiting the feeding of an invertebrate pest by utilizing an aryl-1,4-dialk(en)yl piperazine compound, classified in 514/255.

VII. Claim 28, which is drawn to a method of inhibiting the feeding of an invertebrate pest by utilizing a bicyclic cocaine derivative, classified in 514/299.

VIII. Claim 29, which is drawn to a method of inhibiting the feeding of an invertebrate pest by utilizing a tricyclic antidepressant compound, classified in 514/656 and in other subclasses depending on the structures of the various tricyclic antidepressant compounds.

IX. Claim 32, which is drawn to a method of delaying the maturation of a juvenile invertebrate by utilizing a chloroethylphenylamine compound, classified in 514/646 +.

X. Claim 33, which is drawn to a method of delaying the maturation of a juvenile invertebrate by utilizing an aryl-1,4-dialk(en)yl piperazine compound, classified in 514/255.

XI. Claim 34, which is drawn to a method of delaying the maturation of a juvenile invertebrate by utilizing a bicyclic cocaine derivative, classified in 514/299.

XII. Claim 35, which is drawn to a method of delaying the maturation of a juvenile invertebrate by utilizing a tricyclic antidepressant compound, classified in 514/656 and in other subclasses depending on the structures of the various tricyclic antidepressant compounds.

Claims 1-2,7-8 and 13-18 are generic to Invention Groups I-IV. Claims 23-25 are readable on Invention Groups V-VIII. Claims 30-31 are generic to Invention Groups IX-XII. Therefore, these claims shall be searched inasmuch they read on the elected invention(s).

Within each of the "super" groups [I-IV], [V-VIII] or [ix-xii], the inventions clearly lack unity under PCT Rule 13 since each invention therein utilizes chemically distinct class of compounds. Furthermore, each of the "super" groups lack unity relative to each other under PCT Rule 13 since each of these groups is drawn to a different method of affecting an invertebrate. These different methods are of separate inventive concept and a different field of search would thereby be required for each different method of affecting an invertebrate. For example, the field of search for invertebrate pest control (i.e. "super" group [I-IV]) would essentially be limited to the pesticidal arts, but the field of search for the other two "super" groups, the method of inhibiting feeding of an invertebrate and method of delaying the maturation of a juvenile invertebrate would encompass disciplines of specific invertebrate physiology. Therefore, even though the inventions of the different "super" groups may be classified in the same classes and subclasses in U.S. patent classification (e.g. Invention Groups I, V and IX share the same classification), separate literature search field would be required for the inventions of each of the different "super" groups.

Therefore, for the reasons of chemical distinctness, separate inventive concept and different field of search which would be required, the twelve inventions as set forth above lack unity under PCT Rule 13.