SENSITISATION: CLUE TO THE PATHOLOGY PATHWAY OF CHRONIC ORGANOPHOSPHA TE POISONING
A REVIEW OF THE EVIDENCE WITH CASE REPORTS

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Summary

The marker of chronic OP poisoning is sensitisation : a heightened sensitivity to low exposures of the initiating agent and cross sensitisation or multiple chemical sensitivity (MCS) to a range of unrelated chemicals, including other pesticides, solvents, perfumes and certain foods. Sensitisation can be defined as chemical kindling and is intertransferable with experimental electrical kindling. Kindling is an amplification process where the initial stimulus has no effect. but repetitive stimulation of a brain area, usually in the limbic system induces abnormal discharges and release of glutamate at high frequencies, mediated by N-Methyl-D-Aspartate (NMDA) receptors. Electrical kindling culminates in seizures. Chemical kindling induces dysfunction of sensory reception and emotional responses via the amygdala. impairment of memory and cognition via the hippocampus and interference with odour discrimination via the olfactory lobe. Alterations in motor, endocrine, autonomic and immune function are relayed through the hypothalamus, midbrain and brain stem.

Corticoid releasing hormone (CRH), released in response to stress is essential to the amplification of the excitatory stimulus. Chemical kindlers powerful enough to induce a biochemical stress releasing CRH include GABA antagonists, such as the convulsants bicucullin and picrotoxin, excitatory amino acid (EAA) agonists, opiod peptides, certain chlorinated hydrocarbons, such as dieldrin and lindane, local anaesthetics, cholinergic agonists and acetyl cholinesterase (AChE) inhibitors, notably the organophosphate (OP) and carbamate pesticides.

Kindling is time-dependent ie. time is required between each electrical stimulation or chemical exposure to allow build-up of after-discharges. Hence chemical sensitisation is not due to cumulative toxicity, which would correspond to continuous stimulation and lead in the case of poisons to acute toxicity, or sometimes in the case of toxic irritants to adaptation.

Sensitisation and cross-sensitisation are initiated in the olfactory lobe. OPs are volatile in hot weather or when carried by evaporating solvent. Volatile molecules that by-pass the blood vessels in the nasal epithelim have rapid access through the olfactory lobe via transneural transport to most areas of the brain: the cortex. the limbic structures where repeated exposures to low levels induce chemical kindling, and to all of their connections. In the olfactory lobe itself the first and second order neurons are dominated by excitatory ACh whose role is to control CRH and glutamate release by activating inhibitory GABA. In the presence of OP there is down-regulation of cholinergic receptors and inhibitory control may be insufficient to prevent a CRH-amplified release of glutamate. The result is an intolerant response to certain odours.

Cross sensitisation occurs because short chain volatiles such as formaldehyde, acetone etc. stimulate release of glutamate. Hence those with MCS react to solvents, perfumes or any substance containing short chain volatiles with a hypersensitive release of glutamate and onset of symptoms similar to those induced by the OP itself.

Since 1951 to the present day, the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Health (DoH) have put pressure on farmers to wear protective clothing and face shield. They have dismissed and continue to dismiss the risk of OP poisoning by inhalation, recommending a respirator only in special circumstances. But as the chronically poisoned themselves maintain, inhalation is the major route to dysfunction of olfactory reception and gives direct access to the CNS. Those who absorb OPs via the skin are protected (i) from peripheral paralysis and lung collapse by liver detoxification mechanisms, except certain individuals who are vulnerable due to genetic mutations in liver enzymes and/or to nutrient deficiencies. (ii) from neurotoxicity by the blood-brain barrier except in episodes of stress when there can be a temporary disruption of its impermeability.

Sensitisation pathways are very similar to those inducing long term potentiation (LTP), but there the activity of excitatory glutamate has another objective and is regulated to meet the demand of the neural network for information storage. In the amygdala the unregulated high frequency release of glutamate leads to anxiety, irritability, withdrawal and other forms of emotional disturbance. In the hippocampus it induces a change in opiod gene expression with an increase in the ratio of excitatory enkephalins and inhibitory dynorphins, one of whose effects is poor short term memory. Raised levels of corticoids in the hippocampus induce fatigue and depression.

Chronic OP poisoning has been postulated to be a disorder of cholinergic transmission. AChE

inhibitors raise levels of acetylcholine (ACh) inducing a depolarisation block to which the body responds by down-regulating postsynaptic cholinergic receptors and choline acetyl transferase (ChAT) synthesis. Underfunctioning ACh and failure to build sufficient charge induces non-depolarisation block. Evidence for the rapidity with which this occurs and its persistence over months was given by the increase in muscular junction jitter values in healthy volunteers exposed to sarin vapour .

There is also evidence of an up-regulation of specific post-synaptic cholinergic receptors in the central nervous system (CNS). Alzeimers disease and impaired memory are symptoms of dowo-regulation. But therapies and clinical studies designed to stimulate ACh synthesis or to activate its receptors via ACh agonists or AChE inhibitors induce chronic fatigue, depression and anxiety, suggesting an up-regulation and hypersensitivity of specific receptors. Studies of a line of rats bred to be sensitive to the OP diisopropyl fluorophosphate found increased synthesis of ACh in the cortex and raised numbers of receptors in the hippocampus and striatum. It is clear from the evidence that the cholinesterase inhibitor pesticides induce chronic neuropsychiatric disorders in exactly the same manner as experimental drugs, with identical disturbance in acetylcholine transmission.

Two cases are reported where incorrect therapies were given based on the assumption of a uniform down-regulation : 1. Gallanthamine, an AChE inhibitor was administered in an ME trial to raise ACh levels. One recipient was a farmer's son with symptoms of chronic OP poisoning. His depression deepened and he committed suicide. 2. Suxamethonium, an anaesthetic adjuvant and cholinergic agonist was administered to an otherwise fit elderly sheep farmer in a hip operation, ever since which he sank into a state of lethargy and helplessness. Although not overtly sensitised he had been dipping sheep for many years and suxamethonium was a known risk that should have been avoided.

It is urgent to draw attention to another form of cross-sensitisation – the potentiation of pyrethroid toxiicity by OPs. This has been demonstrated in experiments on hens where a Gulf war combination of pyridostigmine vaccine and permethrin insecticide induced hyperexcitability, tremor and locomotor dysfunction, while each given separately had no effect. Pyrethroids activate glutamate via excitation of Na + channels and thereby act synergistically with AChE ,inhibitors, although this may only be clinically expressed in sensitised individuals with a lowered threshold for glutamate. The worry is that farmers and pet owners are switching from OPs to pyrethroids, and local authorities are recommending that children be treated for headlice alternately with malathion and pyrethroid, unaware of the risks to those who may be OP sensitised. I quote 6 cases - two of them known to me - where OP sensitisation followed by pyrethroid exposure led to acute pyrethroid toxicity, and in cases of chronic OP poisoning to a worsening of symptoms.

There is a pressing need far objective tests to support victims in the claim that their illness is due to OP poisoning. An example is cited of a sheep farmer whose case was time and again dismissed by medical specialists/ psychiatrists until confirmed by Dr. Robert Davies after seven years of searching. A variety of objective tests can now measure the malfunction of tissues damaged by OP toxicity.

Re-exposure of chronic OP sufferers to test their 'supposed hypersensitivity' as *proposed by the joint Working Party of the Royal College of Physicians and Royal College of Psychiatrists is dangerous, unethical and could be lethal.*

Sufferers from MCS can have no confidence in the joint Working Party who deny its existence, although it is widely accepted in US. MCS triggers are best identified under the supervision of skilled practioners, and for severe cases in an Environmental Unit. The offending chemicals must then either be avoided or food intolerances can be overcome with one of two low-dose techniques : (a) enzyme potentiated desensitisation (EPD) (b) neutralisation.

Symptoms of depression and anxiety induced by OP toxicity are conventionaly treated with anti-depressant and anxiolytic drugs. These target the noradrenaline-doparnine-serotonin pathways and ignore the cause : dysfunction of the central sensory system with distortion of sensory responses and maladaptation of emotional ones. It would be helpful to both treatment and research if toxicants were treated as a now emerging third class of disease-causing agents. Microbes were the first discovered class and carcinogens the second.

Research should focus on the clinically ill. Animal models may be misleading *in vivo* and their techniques are unethical. *In vitro,* molecular pharmacology requires human tissue for correct mapping. Concerted efforts should be made to discover new techniques applicable at the molecular level and at the level of the organism. I suggest that the pitfalls of reductionism could be avoided if homoeopathic provings of toxins were used to provide a holistic picture from which pathways might be deduced. The same technique applied *in vitro* might help identify the long term effects of

toxins.

A global ban on the production of OPs and of all chemicals damaging to the environment and to health is proposed, and a moratorium in which (i) the UK Government admits culpability on behalf of the licencing and advisory bodies and gives generous compensation to the afflicted (ii) there is a new culture of research, publicly funded and supporting ecologically sound systems of pest control.

1. Introduction.

The victims of organophosphate (OP) poisoning have pleaded with medical practitioners to confirm what they themselves know, that the illness which afflicts them and its characteristic symptomology is due to exposure - usually repeated - to OP pesticides. Without that diagnosis they cannot claim industrial benefit or if self-employed, bring a lawsuit to compensate for their disability, perhaps loss of livelihood, or be given recognition and respect for the genuineness of their complaint [1,2].

Doctors and psychiatrists claimed that symptoms of psychological distress were proof of a psychiatric illness, treatable with drugs and psychotherapy [3]. Toxicologists claimed the reports were anecdotal: epidemiological studies were too few to prove a connection. The onus has been put on the victims themselves to prove it. The recent report of a joint Working Party of the Royal College of Physicians and the Royal College of Psychiatrists [4] on the "clinical aspects of long-term low dose exposure to OP sheep dip" concluded that in view of "the general lack of knowledge about the causes and mechanisms of the condition.....there was no need to presuppose a particular aetiology so long as the symptoms were treated" (paras 7.9, 7.4). This paper attempts to demonstrate that the symptoms are a direct consequence of the OP exposure. They cannot be isolated from it as “a vicious circle of fatigue, sensory symptoms and disability which then becomes self-maintaining long after the departure or resolution of the original precipitant”(para.7.4.) The symptoms must be analysed in terms of the disturbed biochemical pathways created by the OP toxicity which the body is powerless to correct.

2 Sensitisation : the marker of chronic OP poisoning

The characteristic symptom - the marker - of the chronic OP poisoning syndrome is sensitisation: a heightened sensitivity when re-exposed to OPs at extremely low concentrations. For persons mildly affected, and familiar to thousands of otherwise healthy sheep farmers if they go near dipped sheep, there is a temporary return of 'dipper's flu'. For those with what Jamal has defined as 'chronic OP-induced neuropsychiatric disorder' (COPIND) [1 rev! there are full-blown disabling symptoms. Moreover, in the chronic case, sensitisation to OPs induces a cross-sensitisation to unrelated chemicals, including other pesticides, solvents, adhesives, paints, perfumes and anaesthetics. This is known as multiple chemical sensitivity (MCS) (5] and can be accompanied by various food intolerances or non-IgE mediated allergies, particularly to wheat and dairy products. Chemical sensitisation has been defined as cacosmia [6] "an altered sense of smell and feeling ill" with nausea. headaches, dizziness, memory deficits and emotional lability from certain chemical odours that do not trouble others. Bell et al. [7,8] have proposed that cacosmia is a chemically induced, time-dependent sensitisation or chemical kindling. It is inter-transferable with electrical kindling [9]

Experimental electrical kindling has been used to study epileptic seizures which are associated with the high frequency discharge of impulses. It is an amplification process where the initial stimulus has no effect but repetitive stimulation of a brain area in the live animal or in brain slices induces abnormal discharges of high frequency whose end-point is seizure [9,10]. Chemical kindling is an amplification process with identical high frequency discharges, where intermittent exposure to the stimulant, at a dose which initially has no or minimal effect, induces alterations in neuronal, endocrine and autonomic function [8]. It too, though rarely, culminates in seizures. Its inter-changeability with electrical kindling indicates that the pathway is the same: stress-initiated and maintained by a high frequency release of excitatory amino acids (EAAs), usually glutamate, mediated by NMDA receptors [10].

Evidence indicates that chemical seizures are not due to a cumulative toxicity [11]. Hence it is incorrect to propose that sensitisation is due to small increments of poison. Cumulative toxicity would amount to continuous stimulation and in the case of OPs, to acute poisoning. It is the small increments of *time* [8] between each electrical stimulation or chemical exposure that allows the build-up of after-discharges and of the high frequencies at which hyperexcitability is induced, although occasionally a single acute exposure will induce sensitisation.

In some circumstances continuous stimulation may lead to adaptation and tolerance [9]. Speculatively I propose that a continuous exposure induces either receptor down-regulation or up-regulation of some inhibitory mechanism, whereas an intermittent exposure gives insufficient stimulation for the induction of transcription factors necessary to induce the down-regulation or the synthesis of an inhibitory pathway. Adaptation to continuous exposure might be the explanation for 'masked' food allergies, opiate and alcohol tolerance etc. The unpleasant symptoms of withdrawal would follow from an up-regulation of the receptors or a down-regulation of the inhibitory mechanism, with restoration of glutamate hyperexcitability in either case.

3. Sensitisation pathways

I. The hypothalamic-pituitary-adrenal stress axis

The first symptoms experienced by OP sensitised persons on re-exposure to OPs or across sensitising agent are flu-like symptoms that may include muzzy head, streaming eyes, dizziness, headache, blurred vision, lethargy, aching muscles, tight chest, nausea, diarrhoea, abdominal pain, tingling. The immune system may be depressed but there are no viral antibodies. Behavioural changes include: anxiety, panic, anger, memory impairment, depression. Some may become impulsively suicidal.

The stress-induced excitation of the endocrine, autonomic and peripheral nervous system is mediated by the release within seconds, of the stress hormone corticotrophin releasing hormone (CRH) [12 rev]. Stress conveying signals rapidly activate immediate-early -genes in CRH-expressing neurons of the central nucleus of the amygdala [13 rev]. This is associated with CRH expression and secretion from the hypothalamic neurons of the paraventricular nucleus (PVN) [12], activating the release of adrenocorticorticotrophic hormone (ACTH) from the anterior pituitary. ACTH stimulates the adrenal cortex to secrete corticoids, notably glucocorticoids. In normal circumstances ACTH and glucocorticoids regulate their secretion by feedback inhibition of the release of CRH. However extreme stress induces up-regulation of the CRH gene in the central nucleus of the amygdala and the negative feedback is insufficient [13]. The excitability of the hypothalamic-pituitary-adrenal (HPA) stress axis is driven by up-regulated catecholamines secreted from brainstem neurons, transmitted either directly to the PVN or indirectly via amygdaloidal and other nuclei. Hippocampal stimulation however is inhibitory of HPA activity [13]. Fig.l summarises the effects that a hyperexcitability of the HPA stress axis inflicts on the individual. Specifically we note:

(i) There are CRH-l receptors in the olfactory system [14 rev]. CRH induces opiod release and opiods stimulate the release of histamine from mast cells [81. Inhalation of the stressful stimulant and release of histamine from the nasal epithelium could account for the heightened nasal reactivity in the sensitised. and the fact that within seconds some agent is absorbed by the nasal vasculature into the blood stream, causing the streaming eyes, muzzy head, headache and flu-like symptoms.

(ii) Stress would induce release of histamine from brain stem neurons and their activation of H_1 receptors [15,16]. The dizziness could be due to histamine acting on H_1 receptors (R) in the vestibular nuclei, and the nausea to the combined effect of this and of histamine acting on H_1-Rs and raised levels of ACh acting on muscarinic receptors in the nuclei comprising the vomiting centre.

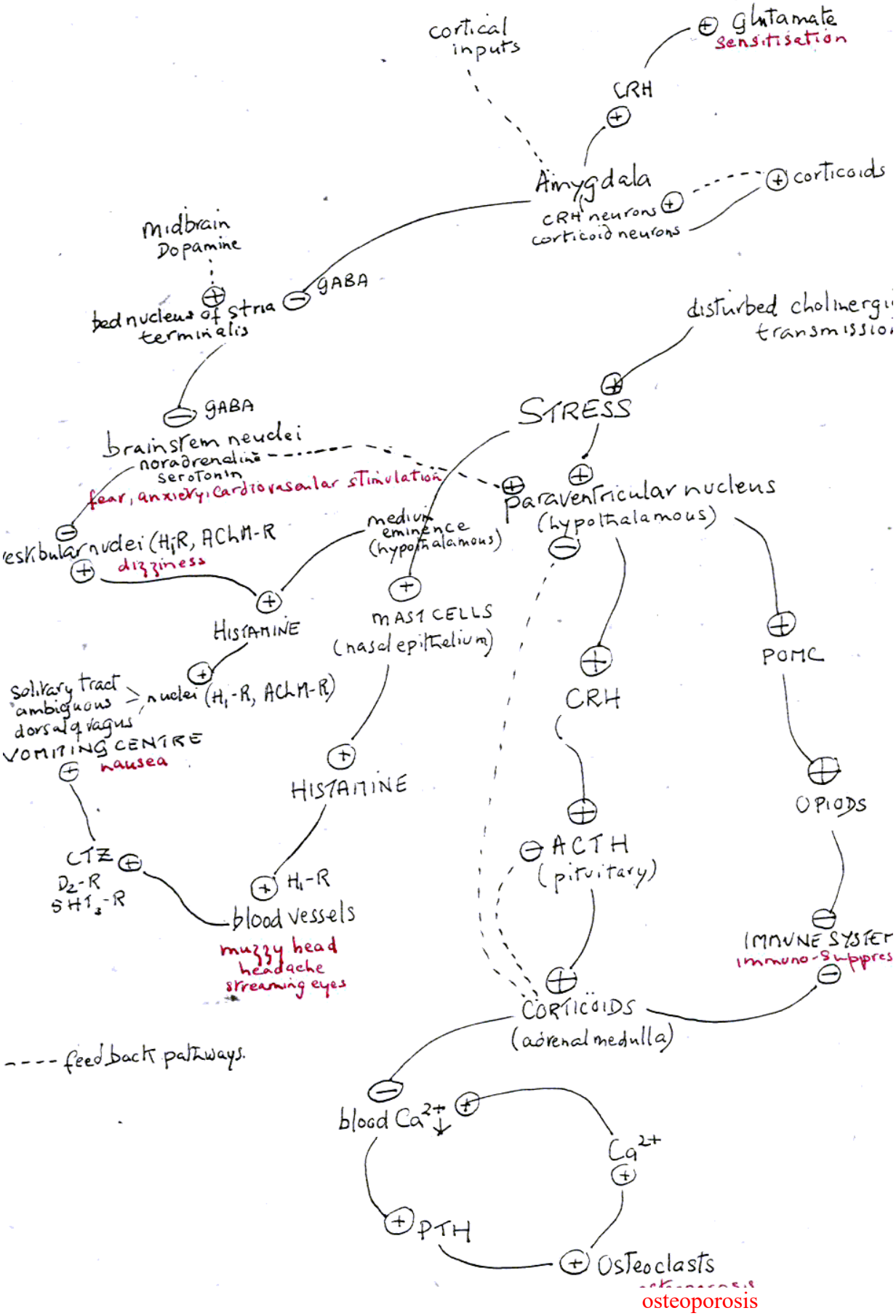
(iii) Opiods could induce immuno abnormalities via their receptors on immunocytes and macrophages [17]. They are one of the triggers in cross-sensitisation and are present in wheat and dairy products [8] which are usually the first items to be avoided in MCS and food intolerance.

(iv) It has been reported that some of those suffering from COPIND have developed osteoporosis. [18]. Dr. Sarah Myhill has about 100 OP sufferers on her books and X-rays have shown a significant number of these have microfractures in their vertebrae (pers. comm). Glucocorticoids suppress Ca^{2+} uptake from the GI tract and increase Ca^{2+} excretion from the kidneys, inducing a Ca^{2+} deficiency that stimulates parathyroid hormone (PTH). PTH activates osteoclasts to demineralise bone and restore calcium levels to the blood stream. It can be expected that in the course of time the continued stress will reduce bone density.

(v) Jamal [1] finds it puzzling that no effects of chronic OP poisoning on the autonomic system have been explored. He reports a consistent pattern of autonomic dysfunction in a group of farmers with COPIND and is studying the underlying mechanisms [19].

Fig. 1 Hypothalamic – Pituitary – Adrenal Stress Axis

Some responses to organophasphate poisoning



II. Stress pathways in the limbic system

Sensitisation is mediated by the kindling mechanism acting on limbic structures. The limbic structures most sensitive to kindling are the amygdala, hippocampus and olfactory lobe [7]. Its pathways depend on NMDA receptor activation and hence are very similar to those inducing long term potentiation (LTP). LTP is a form of activity-dependent synaptic plasticity, proposed as the cellular basis for learning and memory, and is measured as a sustained increase in the post-synaptic potential of hippocampal CA1 neurons or dentate gyrus neurons after delivery of a high frequency tetanic stimulus [20 rev]. It is maintained via cooperative inputs from the local and global dendritic network and with post-synaptic contributions [21]. Electrical and chemical kindling and LTP all involve the high frequency (usually up to 100 Hz) post-synaptic release of glutamate via activation of NMDA receptors. Neither kindling nor LTP can be induced other than by a high frequency stimulation. Activation of NMDA receptors is not by itself effective even where depolarisation is positive enough to remove Mg^{2+} block: or where hyperpolarising K^{+} currents are inhibited or where the release of synaptic GABA is antagonised [22]. But any agent that sufficiently increases the conductance of the NMDA receptor channel will increase the probability of LTP or of kindling induction [21]. This does in fact often occur when GABA is disinhibited *but only in the presence of CRH.*

CRH is now known to be the crucial mediator that amplifies the excitatory stimulus inducing high frequency release of glutamate in the amygdala, hippocampus [12] and olfactory bulb [14]. Its role, once thought restricted to that of releasing pituitary ACTH has now been established (a) as the activator of LTP ie. the means by which sensory learning is believed to be stored in the hippocampal memory map of the young and adult animal. (b) As the key inducer of excitability in the developing brain [12], where the infant animal, mainly via amygdaloidal structures, learns about the world from sensory stimuli for identification of food and arousal of emotional attachment, fear, aggression. (c) As the neurohormone responding to stress. Administration of CRH induces behaviouraI, neuroendocrine and autonomic changes in the experimental animal, similar to those seen after chemical and electrical kindling, and CRH receptor antagonists can abolish stress-induced behaviour [12].

However while LTP and its counterpart long term depression (LTD) have conferred an evolutionary benefit on the conscious animal, kindling whether environmentally or experimentally induced is totally destructive.

III. Limbic connections

The following outline is necessarily simplistic. Sensory information is assembled in the limbic system, with higher order processing in the cortex. A large cluster of CRH-expressing neurons is located in the central nucleus of the amygdala. Stress conveying signals activate immediate early gene (IEG) transmission in these and other amygdaloidal neurons [12], inducing a CRH release which is associated with its expression in the hypothalamic PVN. The central nucleus receives sensory information from the olfactory system and other descending cortical inputs, and ascending thalamic, brainstem and midbrain inputs. Sensitisation to OPs produces chemical kindling. But unlike localised electrical kindling, the malaise spreads further into the the CNS and via connections to brainstem neurons and the HP A stress axis to the autonomic and immune systems.

Major projections of the central nucleus are to the bed nucleus of the stria terminalis, the midbrain central gray nucleus, the substantia nigra, the nucleus of the solitary tract, the raphe and locus caeruleus nuclei and the vagal nucleus. Hence the CRH-expressing neurons in the central nucleus of the amygdala project primarily to the autonomic system [23], and to neurons secreting dopamine, serotonin and noradrenaline, for control of emotional feeling. The major inhibitor of autonomic and aminergic activity is GABA. In this two way system the central amygdaloidal nucleus is in turn directly or indirectly activated by noradrenaline and dopamine and by a range of neuropeptides [14].

The majority of amygdaloidal CRF neurons contain glucocorticoid receptors [14]. While glucocorticoids exert a negative feed back on CRH expression in the PVN and hippocampus, they activate by positive feedback CRH expression in the amygdala [12,13]. Stress increases tyrosine hydroxylase mRNA for noradrenaline and dopamine synthesis [14], necessary for overcoming stress effects and to combat or escape the origins of the stress it increases tryptophan hydroxylase mRNA for serotonin synthesis [14], raising heart rate and blood pressure: a response that may be over-ridden by the reduced serotonergic transmission associated with depression.

Androgen and oestrogen mRNA and receptors located in the central and other amygdaloidal nuclei are associated with the modulation of stress-related autonomic and behavioural responses in male and female [14]. Twice as many females as males report depressive illness and the ratio of female to male MCS patients reaches 4:1 in some studies. [24], providing evidence for the important role of sex hormones in limbic system projections.

The effects of high frequency glutamate release are most easily worked out in the comparatively simple system of the hippocampus. CRH is expressed in GABA interneurons whose dendrites and axons synapse on the granule and pyramidal cells, strategical1y placed to modulate CRH -enhanced glutamate excitability [12] and opiod synthesis, important in the induction of LTP [25 rev]. The high frequency release of glutamate induces immediate early genes, followed by late response gene transcription, with up-regulation of enkephalin mRNA and down-regulation of dynorphin mRNA. An increase in the ratio of excitatory enkephalin to inhibitory dynorphin in the granule cells and mossy fibres of the trisynaptic perforant pathway increases the excitatory activity. Enkephalins act on ų-receptors coupled to Ca^{2+} influx and on δ-receptors coupled to-repolarising K^{+} -channels which they suppress. Dynorphins on the other hand are inhibitory. They activate K^{+} -channels via κ-receptors.

LTP is induced by a complex network of cell signals controlling gene expression. But a sustained shift in opiod gene expression from dynorphin to enkephalin initiated by CRH will result in prolonged hippocampal excitability .Hence in ischaemia or in adult status epilepticus there is neuronal death; in experimental kindling there are hippocampal seizures; and in chemical sensitisation there are injured neurons, with loss of memory and cognitive skills.

Raised levels of glucocorticoids are also damaging. The hippocampus has higher numbers of gluco- and mineralocorticoid receptors than any other brain structure [13]. Hence if stress levels remain high and the elevation of corticoids is prolonged, there may be hippocampal degeneration. Imaging studies show a reduction in hippocampal volume in elderly individuals with high cortisol levels [26]. This may signify memory impairment and/ or depression. Prolonged cortisone administration was discontinued several years ago except where no other course seemed possible. when it was found to induce a deep depression. This explains why in the OP-poisoned a major reaction to the raised hippocampal corticoid levels is likely to be depression. Pharmacologically, depression can be induced by high doses of antagonists to NMDA receptors [10], whereas GABA modulation of glucocorticoid secretion has been identified as a mitigating factor in depressive illness [13]. Since depression is a major reaction to the raised corticoid levels and since in the hippocampus gIucocorticoids exert a negative feedback on CRH release, I suggest *that depression is induced by any mechanism which directly suppresses hyperexcitability.*

4. Chemical Kindlers

Chemical kindlers must be powerful enough to induce a biochemical stress that releases CRH. Experimentally they include [11 rev] GABA antagonists [27] EAA agonists, opiod peptides [28], local anaesthetics [9], chlorinated hydrocarbons [29], cholinergic agonists [30], cholinesterase inhibitors [31]. Among GABA antagonists are the convulsants bicucullin that binds competitively to the GABA receptors reducing chloride permeability and picrotoxin that blocks the Cl-channel. Some, chlorinated hydrocarbons are also GABA receptor antagonists, binding competitively to the t-butyl bicyclophosphothionate site [11]. Gilbert and others have been interested in the effects of low level pesticide exposure on the CNS and studied chemical kindling by endosulphan, dieldrin, lindane and endrin. All were shown to lower the threshold for the induction of seizures in

experimental animals and to be interchangeable with electrical kindling.

The local anaesthetic lignocaine in its analgesic mode blocks Na^+ channels and prevents them conducting. It has the property of 'use dependence' : the drug binds cumulatively with each depolarising pulse, increasing the degree of block and anaesthesia [32]. Should it be administered intermittently the block has no time to build. This explains why local anaesthetics are paradoxically also CNS stimulants and can be a hazard clinically, producing agitation, confusion and a tremor that may progress to convulsions. It also explains why sensitised OP sufferers experience a return of symptoms if given a local anaesthetic. Chris Lloyd SRN who was a nurse in the Gulf war from which she returned totally poisoned, was ill after a dental anaesthetic and would never have another. General anaesthetics whose mechanisms are less well known are even more to be avoided. When Enfys Chapman broke her arm she knew from experience to avoid anaesthetic of any sort and had it set without one. OP sufferers Mervyn Rees, Richard Turnbull and Mrs. June Adams all deteriorated after a general anaesthetic. Mrs. Adams developed ME.

5. Entry pathways of organophosphate toxicity : inhalation - the neglected hazard

Why, it should be asked are there thousands of sheep dippers and crop sprayers etc. who suffer no clinical symptoms? I suggest the answer lies in the fact that (a) the wrong route of entry has been identified as the critical one and (b) there is a varying capability in individuals to detoxify the OP and its degradation products.

Organophosphates enter by inhalation, through the skin, or by ingestion. For years the licencing and advisory bodies have maintained that cutaneous absorption is the major route in OP poisoning [33]. All the advice on protective wear from the 1951 Working party chaired by Lord Zuckerman to the latest updated 1998 Health and Safety Executive (HSE) sheep dipping booklet makes this assumption. In 1951 the recommended protective clothing included "rubber gloves, rubber boots, an eye shield and white cotton overalls, with respirators to be worn in all cases where OPs were handled in enclosed spaces." This advice later became mandatory. The 1998 booklet states that protective clothing must be made of PVC or nitrile (instead of solvent-perishable rubber) and is comprised of bib apron or waterproof coat, gauntlet gloves, waterproof leggings or trousers, wellingtons and a face shield. Respiratory equipment "is not needed" except where pouring concentrate or cleaning up spillage in a confined space or dipping inside a building or working with freshly dipped sheep in still air [34]. But many of the victims are convinced they were OP poisoned by inhaling it. This would particularly be the case if dipping/spraying was done on a hot day with evaporation of the solvent carrier - a practice belatedly advised to be avoided [34].

It is certainly the case in the home for those flea spraying their pets and furnishings, where no advice whatsoever is given. In a hot spell in August 1995 three of my neighbours exposed to Nuvan Top fleaspray vapour were acutely OP poisoned, suffering immobilising skeletal muscle pain, abdominal pain, tight chest and breathing difficulty for several days until by comparing notes they diagnosed the problem. All three responded rapidly to homoeopathic treatment (Physostigmine or alternatively Sheep Dip Nosode). A 4th victim was a lady who replied to my letter in the SW Wales Guardian and had been exposed to OP vapour throughout the summer from flea spray and vapona strips: she slept with a strip above her head and had one in the kitchen. Her GP was puzzled by her chest pains, palpitations, hallucinations and depression. Electrocardiograms found nothing wrong. Fortunately despite that she had developed chronic symptoms, she too responded to sheep dip nosode and supporting remedies. .Nevertheless after weeks of intermittent exposure she had been sensitised and relapsed when in contact with flea collars or solvents in the art classes she ran, necessitating retreatment.

Chronic OP toxicity may be initiated if the OP is sufficiently volatile. It will be volatile if the farmer or farm worker dips or sprays on a hot day, or if it volatilises along with the solvent. Sensitisation depends on how often he inhales the low dose of vapour coming off the wool of his sheep, off his dipped dog or off his clothes. Similarly his wife: she too may have been dipping, washing their clothes and the children playing with the dog. It is via the olfactory bulb, not the skin

that sensitisation is most likely to occur. If the initial mode of entry was cutaneous, stressful conditions would also be required for the OP to cross the blood brain barrier [35 see below]. But a direct pathway from the oropharynx to the brain and hypothalamic and limbic region has been demonstrated in rats, with substances migrating to the brain in minutes via a pathway other than the blood stream [36]. Further studies showed that volatiles contacting the nasal epithelium are widely distributed to the brain via transneuronal transport [37].

The primary pathway for capturing inhaled toxic molecules is the nasal mucosa and its network of blood capillaries. Toxic molecules absorbed into the blood stream are delivered to the liver for detoxification. Nevertheless it only takes a fraction of the odiferous molecules to activate the first order receptor neurons of the nasal epithelium. It is here that recognition first takes place [38]. Olfactory systems are exceptionally similar across the animal phyla, from molluscs to insects, crustaceans, and vertebrates. Hence where information is lacking we can assume the structures and interconnections are much the same [39]: the well-researched locust olfactory system contains about 50,000 cholinergic excitatory receptor neurons. Their axons synapse with the dendrites of the second order projection neurons that correspond to mammalian mitral and tufted cells. These are cholinergic, excitatory, conventionally spiking neurons activating glutamate release from the mitral to the internal granule cells. The presence of CRH-1 mRNA in the olfactory lobe indicates that CRH will enhance glutamate excitability [14], inducing LTP and the synaptic plasticity necessary for storing memories of smell. But as in the amygdala and hippocampus it will induce hyper-excitability when released unregulated under the stimulus of stress.

Modulation of glutamate excitation is effected via dendro-dendritic synapses where feedback and lateral GABAergic inhibition of the mitral cells and other inhibitory outputs allow odour discrimination before transmission of the stimulus to the higher centres of the brain [14,38]. The current model predicts that the inhibition shuts off excitation by weak olfactory stimuli, eliminating unnecessary transmission of information and the confusion of background 'noise'. A major characteristic of the OP poisoned person is their heightened sense of smell. This may be because the GABAergic neurons are insufficiently activated by excitatory ACh due to down-regulation of their post-synaptic receptors. It has been suggested that AChE may play a protective role by enzymatically maintaining ACh at nerve junctions within safe bounds [40], and nowhere more so than in the olfactory lobe where the huge numbers of cholinergic first order neurons indicate that anticholinesterase molecules may first have to saturate this barrier. The proposed rapid and persistent down-regulation of central post-synaptic ACh-Rs suggested by increased neuromuscular jitter values [41 see below] will perhaps have sufficiently recovered by the next dipping or low level OP exposure, in which case sensitisation will not take place. But there are those that only slowly replenish their AChE levels [42] and these may be vulnerable.

Axons of the olfactory bulb neurons extend via the olfactory tract to the lateral olfactory area of the temporal lobe where conscious awareness of smell begins. Extensive connections link the area to most of the brain. An important connection via the perforant pathway of the entorrhinal cortex activates the trisynaptic pathway comprised of the hippocampal granule cells of the dentate gyrus. expressing prodynorphin and proenkephalin genes. and the mossy fibres of the CA3 and CA1 pyramidal neurons. where stimulation of the mu- and delta-receptors induces LTP, or in sensitisation, hyperexcitability resulting in cognitive defects and depression.

How can we account for cross-sensitisation and in the chronically affected the evoking of OP toxicity symptoms by perfume, clothes preserved in mothballs, solvents, new carpets, adhesives. Paints, nail polish remover? The explanation lies in the fact that short chain carbon molecules such as formaldehyde, acetone, methyl ester ketone are EAA agonists. The optimum carbon length provoking glutamate release is 6 or fewer [43,44]. Therefore perfumes, carpet adhesives. paints etc. that contain these volatiles will activate excitatory glutamate in the olfactory lobe [45] with transmission of high frequency impulses down nerve fibres via post-synaptic NMDA receptors. inducing hyper-excitability in those areas made vulnerable by an aberrant ACh transmission. Multiple chemical sensitivity (MCS) will afflict those even when the pesticide sensitisation took place many years before and will continue to do so unless there is a normalisation of the ACh pathways.

6. Variations in detoxification capability

Cutaneous or oral uptake of OPs transfers the OP to the liver via the bloodstream for detoxification and elimination. However individuals vary in their ability to remove the toxic burden and and its metabolites from the circulation. and OPs stored in fat are a potential hazard if released in pregnancy or when losing weight. Should OPs be circulating during a stressful period there is a risk of their entering the CNS. Animal studies have shown that stress induces disruption of the blood-brain barrier [35] allowing penetration by molecules that would otherwise be excluded. The anticholinesterase pyridostigmine is a charged quaternary amine often given experimentally and in clinical trials because it is thought unable to enter the CNS. When prestressed mice were administered an intra-peritoneal injection of pyridostigmine it induced a lowering of brain AChE and an increase in c-fos mRNA compared to controls. *In vitro* exposure of brain slices to pyridostigmine increased electrical excitability and raised the levels of its indirect marker c-fos mRNA. Double blind studies on soldiers vaccinated with prophylactic pyridostigmine during the stresses of the Gulf war showed an increase in CNS symptoms compared to vaccinated peacetime volunteers [35].

The danger of OPs crossing the blood-brain barrier is eliminated if they can be rapidly detoxified. An underfunctioning liver detoxification system may be due to a genetic defect, medical history, inappropriate dietary intake or impaired absorption/utilisation of vitamins and minerals necessary for the detoxification process, anyone of which will contribute to a toxic overload particularly in a polluted environment.

In liver detoxification phase I there are genetic differences in the rate of OP degradation. Paraoxonase (PON-l) hydrolyses the oxon moity of OPs or their metabolites. It is the oxon configuration that makes them potent cholinesterase inhibitors. But the human enzyme is polymorphic at codon 192 and the rate at which the OP is removed from the bloodstream depends (a) on the individual's level of PON-l (b) on their 192 isoform [461. Phase II detoxification depends on linking the toxic metabolite to a water-soluble conjugating group so that it can be excreted. One of these conjugators is glucuronyl that depends on UDP glucuronosyl transferase (UGT). Since UGT-1 uses planar phenols as substrates, it may be implicated in the detoxification of some OPs. Burchell [47] reports that 10 -12% of the UK population have UGT levels about 1/3 of normal, due to a change in the promoter region of the gene, reducing its exp~ession. UGTs are not only important in the liver: an olfactory UGT was identified in the pig, indicating that it may terminate odour signals by clearing the chemical from the olfactory receptors [471. If so and if present in the human, those with lower than normal levels may lose another barrier to sensitisation.

Other important conjugators are glutathione (GSH), glycine and SO_4^{2-} Glycine will be in short supply if there is insufficient folic acid, vitamin B6 and BI2 to override a heterozygosity in the gene whose product is 5,10-methylene tetrahydrofolate reductase and which affects 30 -50 % of the population [48 rev]. GSH and sulphate will be in short supply if there is a deficiency in B6 or a heterozygosity in the gene whose product is the B6-dependent cystathionine ß synthase. The underactivity of this enzyme can be overridden by B6 supplementation at levels 50 - 200mcg/day. These levels are required because B6 deficiency *per se* is usually due not to insufficient intake but to malabsorption or displacement from its enzyme site by a competitive xenobiotic aldehyde. About 1/3 of a healthy British student population was found deficient in functional B6 [49], suggesting that the modem diet, lifestyle or some pollutant in the environment interferes with the uptake of B6.

7. Evidence that chronic OP poisoning is a disorder of cholinergic transmission, and due to a down-regulation or an up-regulation of acetylcholine receptors

There is evidence that a persistent down-regulation of acetylcholine receptors and lowered ACh activity is a major diagnostic marker of OP poisoning. Cholinergic agonists induce depolarisation block. followed by non-depolarising block due to down-regulation of receptors, with loss of activity

at excitatory M_1, M_3 and nicotinic receptors and at inhibitory M_2 and M_4 receptors. AChE inhibitors have the same effect. Exposure to OPs depress AChE and where the enzyme levels are low or slow to be replaced, a post-synaptic accumulation of. ACh induces depolarisation block. The body responds by (i) down-regulating cholinergic receptors and/or (ii) down-regulating acetyl choline transferase (ChAt) mRNA with reduced ACh synthesis. Each of these responses is a safety mechanism against depolarisation block: with loss of transmission, but the inevitable consequence of declining ACh activity and failure to build sufficient charge for cellular activation is non-depolarising block. Hence OP poisoning down-regulates acetylcholine pathways, but should the blood-brain barrier be crossed, the victim develops COPIND. This involves a paradoxical up-regulation of specific ACh receptors inducing cholinergic hyperexcitability in the limbic system. Given below are clinical examples of both.

Binding studies suggest there are many important uncharacterised enzymes other than acetylcholinesterase that are potential targets for OPs. These it is thought might play a role in neurological and non-neurological OP toxicity [1 rev], and if so the parsimonious pathways outlined above would be too simplistic. But since we have no knowledge of the degree to which *in vivo* this binding takes place or whether it can easily be reversed, I have disregarded these potential targets. The-one known exception is 'OP-induced delayed neuropathy' [1] involving inhibition and binding of target neurotoxic esterase. It has long been held the only neurotoxic form of chronic OP poisoning, and is diagnosed by the 'Hen test'. OPs that induce CNS neurotoxicity in hens do not receive a licence. They are therefore irrelevant to the OP-poisoned. Sheep dips commonly contain propetamphos, diazinon or chlorfenvintos; grain in store is treated with pirimphos methyl; agricultural crops sprayed with a variety of licenced products, and in the home dichlorvos and/or fenitrothion are usually in flea sprays. All have been declared non-neurotoxic on the basis of the officially approved 'hen test' whose infallibility was deemed to over-ride the clinical evidence of the victims.

I. Evidence for down-regulation of acetylcholine receptors

(i) Increase in jitter values

Jamal [1] drew attention to a paper by Baker and Sedgwick [41] who reported long term abnormalities of neuromuscular jitter values in 8 normal volunteers exposed to the vapour of very low concentrations of the of sarin (isopropyl methyl phosphonofluoridate) at 0.5 mg/m for 30 minutes. Animal experiments showed the same effect [50]. Jitter values are obtained from single fibre electromyography (SFEMG), in a test that records the action potentials of pairs of muscle fibres. Variations in the discharge between each fibre of a pair gives the jitter values. Jitter increases are the most sensitive indication of impending failure at the neuromuscular junction, occurring long before any clinical muscular weakness. SFEMG is used to detect early and mild forms of myasthenia gravis where autoimmune antibodies deplete the number of post-synaptic nicotinic receptors. As jitter increases, so does the proportion of non-depolarising block, a reflection of the failure of the amplitude of the end plate potential to reach threshhold; Each repeated stimulus reduces the response, since the charge cannot build - a characteristic of non-depolarising block.

There were significant changes in jitter values in 5 of the 8 volunteers at 3 hours and at 3 days post-exposure and at the first long term follow-up of 4 -15 months. Normality had returned by two years. There were shortlived (up to 48 hours) parasympathetic symptoms, red cll AChE inhibition at 3 days and no muscular weakness. An earlier paper [51] had found increased jitter values in patients with the Intermediate Syndrome of OP poisoning. This syndrome, unresponsive to atropine or oxime therapy, has only been recognised in 1987. The authors [41] suggested its explanation might lie either in a reduced number of post-synaptic cholinergic receptors or in a failure of ACh release, either of which would give increased jitter values and indicate non-depolarising block. The Intermediate Syndrome is quite clearly the clinical manifestation of the subclinical jitter abnormalities picked up by the volunteers exposed to 30 minutes low dose sarin. The attempt to treat it with atropine, a muscarinic antagonist could be highly dangerous, because if respiratory

muscles are affected there may be lung collapse [1]. The fact that reactivation of AChE by pralidoxime was not helpful, suggests the problem was not only a depletion of post-synaptic receptors but a reduced pre-synaptic ACh release, probably due to down-regulation of ChAt, brought about to match that depletion. The patient usually recovers in 2 - 3 weeks.

We note from this study :-

(a)That in only 3 hours post exposure there was a down-regulation of post-synaptic ACh receptors and/or of pre-synaptic ACh release.

(b)The down-regulation could persist in healthy persons at least 3-15 months post-exposure.

(ii) Initiation of *Multiple Chemical Sensitivity*

If the exposure to sarin vapour had been followed by further intermittent low exposures, the stress combined with decreased ACh activity could eg. by a disinhibition of $GABA_A$ receptors (52), induce CRH release with amplification of glutamate discharge, sensitisation and production of the symptoms of MCS.

(iii) Memory and cognition impairment

ACh in the hippocampus potentiates LTP. The induction of L TF is associated with an increased synthesis of ß-amyloid precursor protein (APP) [53], which is secreted by different cell types in response to glutaminergic or cholinergic stimulation. Scopolamine, a cholinergic antagonist, renders mice amnesic. APP injected subcutaneously into mice was memory enhancing and overcame scopolamine antagonism. In Alzeimers disease post mortem studies show a decrease in ChAt, AChE and high affinity nicotinic receptors - muscarinic receptors are not affected in Alzeimers - believed to be located on presynaptic nerve terminals. Depletion of presynaptic N-receptors and postsynaptic M-receptors induced 'attention deficits' in experimental rats impairing their performance of cognitive tasks [54].

II. Evidence for an up-regulation of central acetylcholine receptors

An agonist would be expected to improve memory. But here we reach a therapeutic impasse, for the cholinergic agonists arecoline and oxotremorine and the cholinesterase inhibitors physostigmine and diisopropyl fluorophosphate (DFP} consistently induce all the symptoms of affective disorder: depressed mood, fatigue, lethargy and psychomotor retardation [55 rev]. ACh precursors such as choline when used to reverse Alzeimers' memory deficits have in some cases induced depression [56]. Non-invasive nuclear magnetic resonance (NMR) measurements showed that central choline concentration was increased in depressed subjects and reverted to normal levels after successful drug treatment [57]. Cholinergic agonists and cholinesterase inhibitors deepened the depression and fatigue in those with depressive illness or bipolar manic-depression, inducing also hostility and anxiety. The controls too might be affected. In one study [58] 25% of the normal controls reported depressed mood. In another normal controls receiving physostigmine did not become depressed, but only anergic [59], demonstrating that depression and fatigue are not necessarily linked.

How do we resolve the dualism of the conflicting responses of amnesia and depression/fatigue of underfunctioning acetylcholine and its hyperexcitability?

A number of researchers have proposed that acetylcholine is the key neurochemical in affective disorders, while noradrenaline, dopamine, serotonin and GABA play a secondary part. Janowsky et al. point out that an increase in central acetylcholine or stimulation of central muscarinic receptors rapidly induce depressive moods and antagonise manic symptoms. They postulated that muscarinic hypersensitivity or hyperactivity may be a marker of the affective disorders [55].

Cholinergic projections originating in the basal forebrain - specifically the basal nucleus of Meynert and the substantia ionominata - terminate in almost all cortical areas. Studies assessing ACh efflux via these afferent projections have established that increases in ACh release follows the presentation of novel, arousing, sensory, stressful or rewarding stimuli [60], supporting the

hypothesis that the cholinergic system is involved in the detection, selection, discrimination and processing of sensory stimuli, necessary for the formation of memory and retrieval of information. Sarter and Bruno hypothesised that "abnormal regulation of the excitability of cortical cholinergic afferents represents a final common pathway that mediates manifestation of major neuropsychiatric disorders " [60].

Peter Behan's group working from clinical observations and Overstreet's group working with diisopropyl fluorophosphate (DFP)-resistant and DFP-sensitive lines of mice were among those who proposed a mechanism: it was the up-regulation of specific cholinergic receptors that conferred hyper -excitability .

In a study designed to compare by means of neuroendocrine markers the symptoms of chronic fatigue syndrome (CFS) with those of chronic OP poisoning, Behan [61] administered pyridostigmine, a reversible anti-cholinesterase by intravenous injection to 10 male farmers with neurobehavioural symptoms and a well documented history of chronic exposure to OPs. By enhancing cholinergic transmission, pyridostigmine induces secretion of growth hormone (GH) via a disinhibition of its modulator somatostatin. After one hour there was an exaggerated rise in GH compared to that of 10 healthy controls. Pyridostigmine induced a similar exaggerated release of GH in depressives [62] indicating that in both cases there are hyper-active muscarinic receptors.

In a follow-up paper Behan and colleagues [63] compared the effects of pyridostigmine administered to those in the OP study with a clinically similar group of chronic fatigue syndrome (CFS) patients. In both groups, fatigue was the main symptom together with memory and concentration impairment and a shortening of rapid eye movement (REM) sleep latency. Cholinergic agonists and AChE inhibitors induce arousal and awakening from sleep more frequently in depressed persons than in normals and this is ascribed to cholinergic overactivity [55]. The authors hypothesised that the sleep disturbance and neurobehavioural symptoms were due to an abnormality in central ACh transmission in both the patients with CFS and those with chronic OP exposure.

They pointed out that ACh facilitated the release not only of GH but also of CRH from the hypothalamus and suggested ACh hyper-responsivity at the hypothalamic level could be caused either by hyper responsive post-synaptic receptors or a presynaptic autoreceptor subsensitivity. Since it was well known that chronic administration of anticholinesterases could give rise to loss of ACh post-synaptic receptors they hypothesised that a decrease in their number would be followed by a post exposure period of post-synaptic upregulation. Further studies confirmed "the exquisite sensitivity and upregulation of post-synaptic receptors" (no reference given).

Overstreet et al. [64,65] had produced a rat model for research into depressive syndromes. These were the Flinders Sensitive Line (FSL) rat, bred for supersensitivity to the OP diisopropyl fluorophosphate (DFP) and the Flinders Resistant Line rat (FRL) whose behaviour when exposed to DFP differed little from that of normal rats : presumably the normal rats responded with a down-regulation of muscarinic receptors matching that of the FRL rats. Biochemical studies established a significant decrease in brain muscarinic receptors associated with tolerance to DFP [64] and the predicted up-regulation of receptors in the FSL rats was confirmed by the finding of an increased synthesis of acetylcholine in the cortex and higher numbers - by about 20% - of muscarinic receptors in the hippocampus and striatum. No other area was investigated [65]. (Note that Swanson and Petrovich [23] consider regions of the striatum to be part of the central nucleus of the amygdala, receiving inputs from the accessory olfactory bulb concerned with pheromonal communication).

In a stressful environment, the FSL rats were more sensitive to the depressant effects of the cholinergic agonists pilocarpine and physostigmine on locomotor activity, water intake and body temperature than the FRL rats [64]. They were less affected by the antagonist scopolamine, whereas the FRL rats were predictably more sensitive to antagonists. The FSL rats hyper-responsivity to stress led to the suggestion that a propensity to stressability might be a hallmark of depression [55]. But the reduced locomotor activity was only under short exposure (< 1 min) to stressful conditions. Longer exposure (> 1 hour) where the animals became habituated to the conditions resulted in no

difference in activity between them and their FRL counterparts [24]. This is reminiscent of time dependent sensitisation, with short intermittent exposures leading to hyperexcitability, and prolonged exposure to adaptation (Sect. 2) Here perhaps is the clue as to whether supersensitivity to cholinomimetics is a trait (genetic) or state (imposed by circumstances) marker [55]. Clearly it is both, but the FSL rats had I suggest, as speculated in the case of MCS (sect. 2), a predisposition to be sensitised by low dose DFP exposure because some genetic factor for the induction of down-regulation was underfunctioning.

Since the FSL rat was developed as a genetic model for depressive illness, and since MCS is often associated with depression, the researchers postulated that it might prove a model for experimental studies in MCS [24]. This would mean that MCS involves up-regulated cholinergic receptors. I disagree with this prediction. Many of those with MCS are not depressives, particularly where OPs were not the initiating toxin. The pathways to the two conditions are distinct. In MCS it is the NMDA receptor that is up-regulated and the critical cholinergic receptors activating GABAergic neurons can only be down-regulated.

III Hypothesis

To date there is no information on the location of the up-regulated receptors, no direct evidence as to whether they exist in the human or of the mechanism that might produce them. I suggest as a working hypothesis we analyse the pathways producing via the hippocampus chronic fatigue and depression and via the amygdala a distortion of sensory perception which results in mis-judged and incorrect responses.

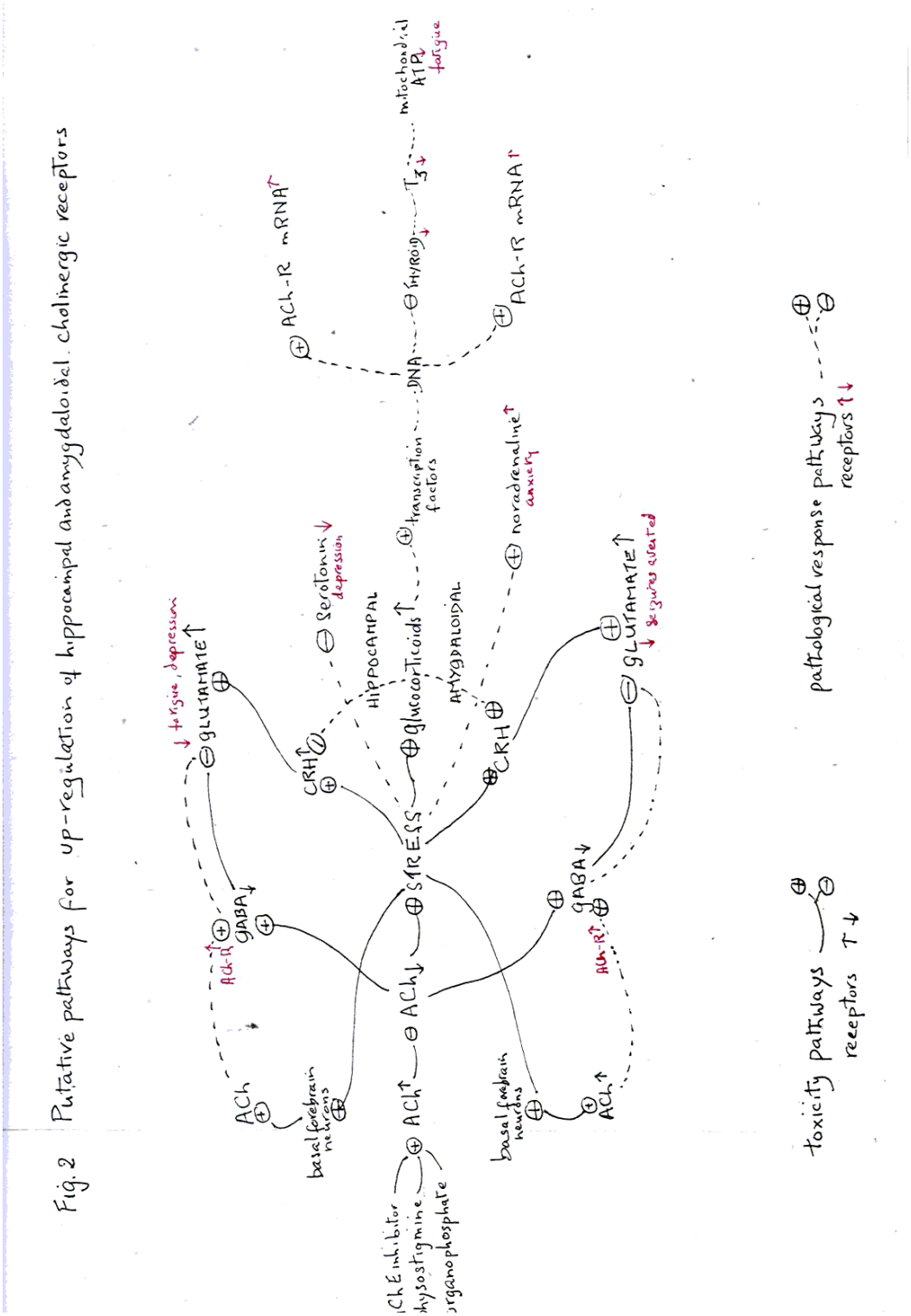
ID the hippocampus : phyostigmine induces lethargy, fatigue and depression.

Non-depolarising block of the ACh excitatory receptors on GABAergic neurons could be the mechanism that facilitates release of CRH. But the hippocampus is characterised by a higher concentration of corticoids than any other part of the brain and glucocorticoids released in response to stress inhibit CRH [13] (sect.4 III), cutting short glutamate excitation and inducing fatigue and depression by some unknown pathway. Stress stimulates ACh efflux from basal forebrain neurons [60], and it might be expected that its raised levels would induce a down-regulation of ACh receptors. But a specific fraction of them are up-regulated, giving rise to hyper-excitability. There is CRH immunoreactivity in hippocampal GABAergic interneurons [12] and since raised levels of corticoids inhibit its expression, *I suggest that* this is linked to correction of GABA disinhibition by up-regulating ACh receptors specifically for that purpose. This, combined with CRH suppression down-regulates glutamate, removing the damaging activity of its hyperexcitability but at a cost. Down-regulation of glutamate contributes to the depression and fatigue.
The lethargy and hypothermia of the FSL rats give a clue to a possible further mechanism contributing to fatigue. I suggest the cholinergic hyperactivity in the hippocampus and amygdala is counteracted by a nuclear suppression of thyroid activity that slows down metabolism and the diminished levels of triiodothyronine (T_3) cuts the overall rate of production of mitochondrial ATP [66], producing the hypothyroid symptoms of chilliness and lethargy. The thyroid status of OP depressives is easily checked. Since chronic fatigue can occur without depression there must be another set of factors acting on the nucleus to induce depression. The efficacy in many cases of the serotonin specific reuptake inhibitors (SSRIs) indicate that these factors involve a down-regulation of the serotonin pathway.

ID the amygdala: In contrast to the hippocampus, glucocorticoids stimulate the expression of CRH. A large cluster of CRH-expressing neurons is located in the central nucleus of the (rat) amygdala [12]. Stress increases ACh efflux, hence the hallucinations and psychotic symptoms due to impaired ability to discriminate and filter sensory inputs, must be induced by an up-regulation of specific ACh receptors and ACh hyperactivity, again for the purpose I suggest of correcting GABA disinhibition. Restoration of GABA function and the switching off of glutamate hyperexcitability may be an even more important safeguard in the amygdala, protecting against seizures which the

raised levels of CRH might have provoked. The anxiety and tachycardia and the hostility and withdrawal symptoms can be attributed to the increased activity of noradrenaline and dopamine respectively in response to stress.

Finally, methyl phenidate reverses fatigue and depression [55,67] by stimulating the release of dopamine and noradrenaline from nerve endings, thereby antagonising ACh. Since dopamine and noradrenaline increase the efflux of ACh from basal forebrain neurons [60], can we deduce that their antagonism is achieved by raising levels of ACh sufficient to down-regulate the hyperactive cholinergic receptors, and normalise the activity of hippocampal glutamate? Recovery from fatigue and depression may still leave the person anxious and/or irritable (see cases 5 sect. 9 and 7sect. 10). Fig.2 summarises these putative pathways.



From the evidence presented it is clear that the cholinesterase-inhibiting organophosphates and carbamates are responsible for inducing neuropsychiatric disorders in exactly the same manner as experimental drugs. The depression, poor memory, psychomotor retardation, anxiety, hostility, chronic fatigue and a host of exaggerated muscarinic responses in the autonomic and endocrine systems follow from an identical disturbance in acetylcholine transmission. To dismiss the neurotoxicity of these OP insecticides as ' unproven' by clinging to the belief that OPs (a) are not nonnally inhaled and (b) do not cross the blood-brain barrier is an unpardonable sophistry. To demand of the victims 'scientific proof' that OP exposure was the cause of their condition is a dereliction of duty.

8. Tragic outcomes of therapy blunders : two cases

Cases I and 2 below are examples of the hypersensitivity induced in muscarinic receptors by cholinesterase inhibitors and cholinergic agonists. They illustrate the extreme care that must be exercised in the treatment of persons that have been exposed to OPs and carbamates, and the importance of understanding the disturbed pathways that produce the symptoms in the chronically poisoned. Failure to do so causes immense suffering and can be lethal. Refusal to do so means that the medical profession allows its members to commit the same blunders over and over again.

CASE 1. Ill effects of gallanthamine

No doubt it was the assumption that persons with myalgic encephalitis (ME) had down regulated ACh receptors that underlay the theory that Gallanthamine would be therapeutic for the muscnlar pains and stiffness and certain neurobehavioural symptoms of ME. Gallanthamine hydrobromide is an acetyl cholinesterase inhibitor, able to cross the blood brain barrier and said to be selective for nicotinic receptors. CFS patients treated with gallanthamine have been reported to show improvement of symptoms [68].

A farmer's son with a history of exposure to OPs and one assumes, diagnosed as having ME, was recruited to a trial studying the effects of gallanthamine in CFS. His depression increased and he committed suicide (Radio 4 Farming Today 9.1.98). Although there is no proof the two events were connected, his occupational history and symptoms suggest he had been sensitised by an acetyl cholinesterase inhibitor ie. an OP. Gallanthamine had deepened his depression. This should have been predicted from the evidence (a) that cholinomimetics can be depressive and (b) available from his medical and occupational history.

CASE 2. Ill effects of suxamethonium on the OP - sensitised.

Dick. Hopkins retired from sheep farming in 1994 after years of dipping against scab and flystrike, without experiencing any of the symptoms of dipper's flu. He was a fit and active elderly man, much in demand to judge at shows. However after a hip replacement operation he developed joint and back pain, at first diagnosed as arthritis, and an out of character anxiety neurosis. This was followed by an inexplicable deterioration during which he became lifeless and lethargic, so that he was virtually confined to bed, since he could not stand without help or sit long in a chair without exhaustion. Although aware, he was unable to hold a conversation. He was permanently drowsy, sometimes confused. He was subject to myoclonus, loss of grip and sudden temperature rises lasting one or two days, usually accompanied by a rash. His first convulsive fit was Sept.I995 and further episodes at approx. 7 weekly intervals necessitated medication. Brain scans revealed nothing wrong. His consultant at first thought it might be a form of parkinsonism, but this was ruled out by a lack of response to treatment and by the fact that at times he was much improved. His family suspected he might be suffering from OP poisoning, supported by the fact he was very ill when the dog was flea sprayed and all his symptoms worsened when he was visited by farmer relatives in their working clothes. On the other hand since his condition seemed to have been initiated by the hip operation, they thought a reaction to the anaesthetic might have been responsible. Everything fell into place when they learnt that suxamethonium potentiates OP

poisoning, and his GP discovered from the hospital records that this was one of the anaesthetic adjuvants he had received.

Suxamethonium is a cholinergic agonist and its prolonged stimulation can produce post synaptic depolarisation block followed on further dosing by down-regulation of receptors and non-depolarisation block. This is normally avoided by its rapid removal by plasma cholinesterase. But if frequent exposure to OPs or some other cause has lowered cholinesterase levels suxamethonium may induce lung collapse, as in two cases known to me: one a farmer sensitised by sheep dip, the other a Gulf war veteran.

Mr. Hopkins took a long time to come round but did not suffer any acute effect, perhaps because he also received atracurium, an antagonist that might have counteracted the peripheral effects of suxamethonium. But his lethargic symptoms and drowsy inattentive state are reminiscent of physostigmine poisoning. It was, I suggest the inadvertant administration of suxamethonium that explains why Mr. Hopkins, a cheerful healthy man who went in for a trivial hip operation, was reduced in a few months to a state of helplessness. It will be argued that suxamethonium has no central effects because it is too highly charged to cross the blood brain barrier. But like pyridostigmine it too could enter if the tight junctions of the blood-brain barrier were temporarily disrupted under stress : a stress imposed by the administration of a cholinergic agonist on one who must have been subclinically centrally sensitised. Despite that the interaction between suxamethonium and acetyl cholinisterase inhibitors is well-known to anaesthetists, no one thought to ask was he a sheep farmer with a history of exposure to OPs.

9.. Potentiation of pyrethroid toxicity by OPs

It is urgent attention be drawn to the potentiation of pyrethroid toxicity by OPs. The lethal effects of their interaction was the bitter experience of thousands of US and UK Veterans returning from the Gulf. In experiments simulating Gulf war exposures, to see whether combinations of two or more agents produced greater neurotoxicity than that caused by individual agents, pyrethroids were found synergistic with cholinesterase inhibitors. One of these combinations was the prophylactic vaccine pyridostigmine and the insecticidal spray permethrin. Pyridostigmine and permethrin administered together orally to hens over a period induced hyperexcitability, tremors and locomotor dysfunction. Given separately at the same dose, each had no effect [68].

Pyrethroids, like OPs can be inhaled or taken up by the skin. They are of low mammalian toxicity but on high exposure symptoms induced by acute toxicity may persist for more than two years, with fatigue from exercise and mental work and other forms of disturbance to the motor, sensory, autonomic and immune systems. Chronic exposure may result in polyneuropathy and personality changes, chemical sensitivity and CFS [69 rev]. Pyrethroids are Na^+ channel agonists evoking repetitive after-discharges [32 pp454-457]. ie. they contribute to nerve terminal excitability .More seriously in the CNS, by increasing conductance of NMDA receptor channels they provide the critical factor essential for glutamate hyper-excitability, the basis of sensitisation.

Examples follow:-

CASE 3. In Behan's trial investigating the effect of pyridostigmine on farmers and workers chronically ill from exposures to OPs [61] (sect. 7 II), it should be noted the researchers did not realise that the dip to which patient 2 had been exposed for at least 3 years was sheep Cypor "spot on", a cypermethrin pyrethroid. His symptoms matched those of the OP-exposed and were in the top rank of severity : fatigue 4+, myalgia 4+, attacks of sweating 4+, depression 3+. Since he had had the illness for 5 years, it can be assumed he switched from OPs to cypermethrin. Hence either his chronic OP toxicity had lost none of its severity, or the cypermethrin toxicity was potentiated by tbe previous two and unrecorded earlier years of OP exposure.

CASE 4. A fit farmer became ill after dipping with the pyrethroid flumethrine. He bad extensive urticaria, abdominal pain, vomiting, malaise and generalised muscle ache that developed into acute, though temporary polyarthralgia. Tests contraindicated viral infection or serum sickness. He had never before been exposed to pyrethroids and previously had only used organophosphates [70]. In

view of the finding [68] that OPs and pyrethroids are synergistic, it can be deduced that he had been subclinically sensitised to OPs, and this potentiated pyrethroid toxicity.

CASE 5. A 58 year-old man was referred to a psychiatrist after an 18 month history of CFS and depression. He reported that his symptoms developed one August after he had been using a pour-on insecticide containing 1% of the pyrethroid deltamethrin. He had not used organophosphate insecticide since the previous April, so the psychiatrist concluded that if his symptoms were a toxic reaction they were likely to be in response to the deltamethrin. An anthelminthic he had also used - benzimidazole - has little or no mammalian toxicity. He said he was a 'changed man, anxious and irritable.' He was treated with fluoxitine and his mood improved, but he lost his job from being unable to work. 4 years after his initial referral he was no longer lethargic but still anxious, irritable and indecisive, and 'not the bright, joking, social person I used to be' [71].

Again it can be deduced that he had been subclinically sensitised by OPs and this potentiated pyrethroid toxicity .

CASE 6. Cone and Sult [72] described a group of casino workers exposed to a mixture of carbamate and pyrethrin insecticides who subsequently developed chronic multi-system symptoms, cognitive difficulties, and sensitivity to the odour of pesticides, perfumes, gasoline, newsprint, and cleaning agents.

CASE 7. John Hywel Williams, sheep farmer had suffered chronic OP poisoning since 1987. Although better now than for the last 12 years, the flexor muscles in his left foot are paralysed, as are his intercostal muscles so that he can only breathe from his diaphragm. OPs made him asthmatic and he cannot go near OP dipped sheep without gasping. He has not dipped with OPs since he deduced the cause of his illness in 1991. 1994 -1996 a contractor dipped his 500 sheep with bayticol, a long acting flumethrin pyrethroid. In contrast to OPs which he can sense for up to a month, the pyrethroid does not stay more than 48 hours on the wool so that after dipping he can work with his sheep. 1997 he dipped them himself with crovect, a cis-cypermethrin. double dipping 14 days apart. Despite that he wore a respiratory mask, it gave him a painful headache and made him sweat. He did not use it for the next 8 months, but Sept. 1998 he cleaned out the crovect from the dip. Next day he was dizzy , unco-ordinated, unable to keep his teet. His backache was worse and flexor muscle paralysis now started in his right foot. He had pains in his forearms and hands, prickling in the skin of his right thigh followed by loss of feeling. He brought up putrid matter from his upper chest. No doubt Mr. Williams' careful note taking would be classed by some as hypochondria and dismissed as a symptom of anxiety, instead of valued for its evidence that here we have another case of OP sensitisation, potentiating a pyrethroid poisoning that would not otherwise have occurred.

CASE 8. Four nurses, one of them Mrs. Barbara Lawson, suffered severe diasablement from working in Broughton hospital (Clwyd Health Authority), where their workplace was treated against cockroach infestation. A new annex had been built but cockroaches were endemic in the old building where the four nurses looked after 'short care' patients. The contractors sprayed frequently, often weekly the patients' rooms, the nurses' kitchen, including drawers of utensils and dishwasher where the cockroaches bred. For years pesticides were inhaled by nurses and patients. No attempt was made to evacuate anyone when spraying was in progress, although as symptoms became intolerable spraying was done only during the night, but fire doors and windows were closed at night in the interests of 'safety'. Mrs. Lawson' symptoms began in 1988. She battled on with many days off work, until one day in April 1992 when the contractors had been in the night before and no one had warned them, completely crippled and walking on sticks she was forced to retire.

Since some of the sprays were known to be OPs the nurses finally deduced that these must be the cause of their lachrymation, headaches, dizziness, ataxia, muscle pains, asthmatic wheezing and airway obstruction, the temporary numbness and paralysis down one side of. the face and 'personality changes'. Mrs Lawson learnt from her medical records that the highly toxic OP parathion had been sprayed at one time. But the nurses' requests for information as to what was

sprayed and on which dates were met with blank refusal. Documentation of spraying from April 1989 -Dec. 1991 was eventually extracted from the hospital after a sit-in with media support in order to give the consultants at Guy's Hospital National Poisons Unit the necessary data.

The printout (enclosed) shows that pyrethroids were used as an alternative to or combined with OPs. Coopex, comprised of the OP coroxon combined with phenothiazine was sprayed April 10 and April 14 1989, followed by 7 pyrethroid sprays (2 permethrin 5 deltamethrin) May 5 to July 18. Cooper's Multispray was used Aug 9. This is a combination of the OP pirimphos methyl and permethrin with a pyrethrum extract. It was followed by deltamethrin Aug 21 and Oct 11. There was a brief respite while rodents were dealt with and deltamethrin was resumed in 1990 - Jan 5 Feb 14 March 6 March 30. A carbamate (Ficam) was sprayed April 4 May 29 June 27. Then Cooper's Multispray July 6, followed *the next day* by Coopex dust (OP coroxon mixed with phenothiazine). Carbamate July 16 and Coopex dust *the next day* July 17 and again July 23 and Aug 3. Sept 27 an OP Iodophenphos. Dec 4 Iodophenphos and Coopex dust together. These records, wrung out of Broughton hospital in the glare of publicity are the only ones available. The nurses were told there was a '16 year list' but were not allowed to have it.

Mrs. Lawson and one of her colleagues have been litigating with Clwyd Health Authority for years, with no redress to date for their disability and loss of livelihood. No doubt there is a reluctance to admit not just liability but the whole catalogue of mismanagement, negligence and cover-up. Broughton hospital including the new annex was pulled down some 18 months ago and already private housing conceals the site and doubtless also some awkward evidence.

Guy's Hospital refused to diagnose or treat Mrs. Lawson and her colleague Mrs. Annette Griffin until Clwyd Health Authority admitted liability in writing. Their blood and urine samples came back captioned 'not analysed'. Guy's Hospital for reasons best known to themselves 'walked by on the other side'. They did however give the two nurses life-saving advice: to go for treatment to Dr Jean Monro who runs Allergy and Environmental Medicine Ltd, Breakspear Hospital.
Thanks to their fighting spirit they managed to get funding. After an intensive course and sustained by a week's treatment every three months, with weekly injections of vitamin B12 both have regained much of their health and strength. Neither are fit for work. Mrs. Lawson cannot walk more than a few steps without exhaustion. She has headaches off and on. There are muscle pains in her legs, and a numbness in the right leg from toe to calf.

It is difficult to tell from this last case how much was due to the OP poisoning and how much to the sensitisation inducing pyrethroid toxicity. *But I would urge COT to examine the synergism between OPs and pyrethroids and on the precautionary principle warn the public and the Health Authorities that pyrethroids could be toxic where there has been a history of OP exposure.* Many of those who used OPs are now switching to pyrethroids. Some may have been sensitised without their knowledge in the course of their occupation. The home is known to be a greater pesticide hazard than the workplace. Hundreds of thousands of children and adults that flea spray their pets, are replacing OPs with pyrethroids since they believe quite rightly they are safer. Local Authorities are advising that children's heads be treated alternately with malathion and pyrethoid to inhibit the development of resistant lice. We already have soaring figures of children with ME whose symptoms match those of chronic OP poisoning [61]. Let us pay heed to the examples cited of OP/ pyrethroid cross sensitisation and not wreck any more young lives. Fleas can be controlled with Program (lufenuron) and headlice removed by the Bug-buster kit developed by Community Hygiene Concern (160 Inderwick Rd. London N8 9JT)

10. Objective testing

One of the major worries of OP sufferers is that they cannot get a diagnosis because they have no way of providing the demanded 'proof ' of a connection between their exposure and their condition.

CASE 7 contd. of John Hywel Williams (sect. 9). He became ill with chronic OP sheep-dip poisoning in 1987 when he was 33 years old, although symptoms started in 1985. He "cracked up psychologically" with anxiety and panic, his "mind in a terrible turmoil". He felt light-headed, "as if his head was detached from his body". Headaches were such he felt his head would explode. He had pains in his testicles, hands, wrists, feet. Painful contractions in his bowels. He could not coordinate: at one time ataxia was so bad he could not walk but kept falling over. He became asthmatic. His GP gave him pethidine which aggravated the symptoms. He had several referrals to psychiatrists and was given drugs for 'nervous debility'. He does not know how he got through the first four years. He was suicidal: "in a black void with no future." But he found if he told himself 'hang on 5 more minutes' he could see one day ahead and that way he survived.

1991 the news of OP poisoning spread among farmers and he realised what was wrong. He took notes of how the dips affected him and stopped dipping, but his symptoms flared whenever he went near dipped sheep. He telephoned the Vet Directorate and was sent a confidential questionnaire by the National Poisons Unit, Guy's and Lewisham's NHS Trust (enclosed). According to the questionnaire he had all the symptoms of OP poisoning, but he told me they took *2½* years to reply. 1993 his GP referred him to see Dr. Virginia Murray at Guy's Medical Toxicology Unit. After a detailed examination where a blood sample was taken, she wrote to his GP 29.7.93 (enclosed): " I suspect that most of Mr. Williams' symptoms possibly relate to his considerable concern about his potential past exposure to organophosphates I would be most grateful if it might be possible to send me copies of any relevant consultations he has made with you so that I can confirm the dates with his exposures." Jan/Feb 1994 Dr. Murray wrote and asked Mr .Williams to supply another blood sample. Anxious for the diagnosis of OP poisoning so far denied him, he gave himself a prolonged exposure to dipped sheep at the market. It made him so ill he could not get to his GP for the sample to be taken. It was after this exposure that he developed paralysis in his intercostal muscles and in the flexor muscles of his left foot. He heard no more from Guy's Toxicology Unit, and still searched for someone to give him a diagnosis.

May 26 1996 he was referred to a Brecon neurologist re the damage to his left foot. The consultant reported "it was difficult to make sense of the left foot story...There was no evidence of neurological disease." 1997 he was referred to Dr. R.D.Davies, consultant psychiatrist for the Avalon NHS Trust, who has devised a simple assessment, identifying 10 characteristics of COPIND which stipulates that a score of 7 or more indicates the syndrome [73]. Dr. Davies confirmed OP poisoning and wrote to his GP Oct.8.1997 (enclosed). "Mr. Williams shows a full house of diagnostic features of organophosphate-induced neuropsychiatric disorder and almost certainly peripheral and autonomic neuropathy and OP related asthma. Added to this however he also has a significant anxiety state. The relationship between this and OP exposure is not quite so clear and certainly is not an invariable feature of OP affected patients. There are however theoretical reasons to suppose that an individual already predisposed to anxiety would be made worse by the exposure."
Mr. Williams is still on prozack for depression and on antihistamines and steroids for his asthma. A spirometry test in early January 1999 at Morriston Hospital showed a maximum volume ventilation (MVV) 78% of normal, indicating a neuromuscular disorder. He has a lot of pain up his legs, spine and round his shoulders. His legs can give way under him. Reflex tests indicate spinal damage. It may turn out he is one of those OP victims recently discovered to have microfractures in the vertebrae (sect.3 I). He is permanently tired. He says: “1 have sheep corning off the mountain to see to, but I have lost my motivation.” Yet he is afraid to retire "because if I did not have work to do I would sleep all day ."

Mr. Williams searched 7 years for someone to give him a medical confirmation of OP poisoning. The same denial on grounds of 'no evidence of a connection' frustrates other OP sufferers. Yet the joint Working Party of the Royal College of Physicians/Royal College of Psychiatrists despite the testimony of those they interviewed still recommend “epidemiological studies aimed at developing

means of quantifying OP sheep dip exposure and relating this to clinical symptoms" (para.9.6), a requirement that will only open the door to endless demands for more exacting 'proof'. Had the Working party ventured out of their committee rooms, they could themselves have questioned the hundreds of mildly sensitised farmers who would identify for them a pen of dipped sheep by the instantaneous onset of flu-Iike symptoms.

What is needed to coofirm COPIND are neurophysiological objective tests that correlate with the symptoms. They could be done on healthy volunteers as in the sarin exposure studies [41], or better still by researchers on themselves, since self-monitoring is more efficient [75]. Tests could be done on mildly sensitised volunteers by briefly exposing them, as they commonly are, to dipped sheep. On *no account should those with chronic OP poisoning ever take part.* This is unethical and dangerous. John Williams responded to such a request and added muscle paralysis to his other symptoms (sect. 9). Others fortunately have refused. The victims' testimonies should be respected. And also their amazing courage.

Objective tests [1] giving evidence of nerve damage include conduction studies [76], quantitative sensory testing with vibrotactile and thermal sensation measurements [76], and the more sensitive measurement of single fibre jitter values [41] indicating peripheral sensory neuropathy. Encephalography (EEG) readings [77] and event related potentials [78], indicating abnormalities in the CNS. Supportive tests would include imaging studies of the hippocampus [25] and assessments of cognitive decline based on the Wechler Adult Intelligence scale [79]. It must be borne in mind that all these tests will show some measure of reversibility and will depend (a) on the person's recent exposure to sensitising agents including food intolerances and stressful circumstances, and (b) on the degree to which their system has recovered or been helped to recover.

The Royal College of Physicians/Royal College of Psychiatrists Joint Working Party refuse to admit the existence of MCS: "the validity of this concept is rejected by most authorities for lack of substantive evidence" [para.4.9]. Who are these 'authorities'? Again no attempt has been made to enquire among the thousands of MCS sufferers who are the best authorities on their allergens and intolerances, nor among the medical practitioners who successfully treat them with Enzyme Potentiated Desensitisation or Neutralisation [80]. The Working Party should have examined developments in United States [81rev. ppI73-193]. 1987-88 the Environmental Protection Agency (EPA) was forced to become concerned when several dozen of its own employees reported MCS following remodelling (recarpeting, painting and furnishing) of its Washington D.C. headquarters. 1991 two federally sponsored workshops were devoted to MCS and advocated further research. 1992 the US Congress asked the Agency for Toxic Substances and Disease Registry (ATSDR) to direct $250,000 from its 1993 budget towards chemical sensitivity and low level exposure environmental workshops, and for the past 5 years ASTDR has fostered sound scientific research in this area. 1994 the Department of Veterans Affairs established 3 VA medical centres to research Gulf veterans' health problems, including MCS. Sept. 1995 the Rutgers University Environmental Occupational Health Sciences Institute and the National Institute of Environmental Health Sciences co-sponsored a workshop in New Jersey entitled "Experimental Approaches to Chemical Sensitivity" to explore various hypotheses advanced to explain MCS.

MCS associated with workers furbishing new buildings and with people living/working in them and also with people living near hazardous waste sites is becoming accepted in US even though the mechanism is not understood. 1990 the National Institute for Occupational Safety drew attention to the fact that neurotoxic disorders are one of the nation's 10 leading causes of work-related disease and injury. But the role of *pesticides* particularly of some OPs and carbamates as initiators of neurotoxicity and of MCS has been ignored by the federal authorities, one suspects under pressure from the heavily compromised Food and Drug Administration (FDA). Not till 1995 did the Environmental Protection Agency (EPA) issue guidelines for evaluating *neurotoxic* risk from chemicals. It was left to individual states to respond to public disquiet: 1994 the California Department of Health Services funded a state-wide population-based epidemiological study of chemical sensitivity and 1994-5 the State of Washington authorised its Department of Labour and

Industries to invest $1.4 million in research on chemically related illnesses including MCS.

In UK, MCS sufferers face the problem of convincing the medical establishment that MCS ie. a heightened sensitivity to certain chemicals and foods is not due as certain experts proclaim, to 'their belief that they have it', to 'anticipatory anxiety', or to 'conditional responses', [para.6.7,7.8]. The matter can be settled not by theoretical argument but by objective testing.

Dudley [43] diagnosed sensitisation by measuring auditory and cognitive evoked potentials in MCS-volunteers before and during exposure to a set of chemical odours and correctly correlated them with those the volunteers themselves reported to be triggers of' an inability to perform the activities of daily living. This experiment might be considered unethical, but the volunteers considered exposure to the chemicals in the lab was no different to what they regularly had to encounter in the workplace.

A confounding characteristic of MCS is adaptation [81 rev. pp34-54l. Repeated exposures to fumes, solvents, certain foods can result in habituation or 'masking' of symptoms. The person may become addicted to certain foods such as wheat, caffeine, dairy products, feeling worse when these are missing from the diet even though they may cause apparently unconnected gastro-enteritic, arthritic or neurological problems. The only way to detect adaptation to one or more allergens is by withdrawal from all suspected agents, during which a person at first feels worse, then very much better with clearing of the deeper-seated problems. Suspected substances are re-introduced one at a time and the offending chemicals or foods identified by a flare up of the previously masked symptoms. (See sect. 2 for a possible explanation).

The most effective investigative procedure for identifying the sources of an individual's MCS so that these can be avoided, or else treated [80] is to isolate the patient in an Environmental Unit free from all forms of toxic pollutant: fumes, dusts, adhesives, paints, tapwater, known ill-tolerated foods; and then to monitor the effects of re-exposure to each. This procedure is practised by clinical ecologists practitioners of environmental medicine. It was pioneered by Theron Randolph in the US in the 1950s, and taken up notably by John Seiner and by William Rea. Rea's Environmental Unit is in Dallas. In UK the Airedale Allergy Centre is a small unit in W.Yorks, whose Medical Director is Dr. Jonathan Maberly.

II. Treatments

The joint Working Party acknowledge (para. 75) that 'the symptoms are real' and must be treated sympathetically and empirically. They do not commit the cardinal error of confusing *diagnosis* with *aetiology*, and eschew the belief long held among doctors and psychiatrists that because patients have a distressed state of mind which often responds to drugs, *ergo* that was the cause of their condition. The Working Party however, run away from the problem by maintaining that it is sufficient to treat the disease and there is no need to establish OP poisoning as the cause or work out the aetiology (para. 7.5).

Their advice on 'exposure and avoidance' (para. 7.8) is extraordinary. '*the effectiveness* of *re-exposure and avoidance reduction in the treatment* of *chronic pain was to be explained'* .They seem to be confusing re-exposure to a lethal chemical with re-exposure to fearful situations. It is well known that after a fall from a horse it is indeed important to 'gain a sense of mastery and control' by getting back into the saddle. This is psychological self-help for overcoming fear. But only a fool would put their finger back into the fire. Only the superior wisdom of a bigger fool would insist they do so.

They give no recommendations for treatment other than with drugs - usually tricyclics,

sometimes SSRIs, targeting the catecholamine - dopamine - serotonin pathways for relief of depression/ anxiety. Some are helped by such treatments, others made worse. By refusing to accept, OPs as the cause the Working Party are impotent in understanding their effect : dysfunction of the central sensory system, with distortion of sensory responses and maladaptation of emotional ones.

In the experience of alternative therapists long term recovery depends on stimulating the system to correct itself. Supportive help is needed for the restoration of basal energy via nutritional supplementation with magnesium, anti-oxidants, B vitamins, co-enzyme Q and essential fatty acids. Unfortunately UK medical schools give no training in mineral/vitamin nutrition and the Working Party are dismissive of their value. The "objective evidence-based justification for their use" (para 5.12) is in the volumes of research mainly American, published in prestigious journals and textbooks, and in the attested experience of those that suffer from OP poisoning and by their help have regained control over their lives. If nutritional and other alternative therapies were available on the NHS via GP referral they would in each case save our over-burdened NHS from the enormous expense of a lifetime on drugs and hospitalisation from drug side effects [82].

12. Research : the proper study of mankind is man

Studies of the clinically ill and the sensitised should be the focus of OP research. It is a matter of concern that COT have interviewed few victims : they have not acknowledged Barbara Lawson's letter offering to give evidence and have ignored John Williams' telephone call. All OP victims should be invited to contact COT and interviewed in person or if necessary by telephone.

Much of the research quoted depends on animal models. But however useful the data it is unsafe to extrapolate from animal models, whether *in vivo* or *in vitro.* Many of the studies quoted were done on rats' brains, but *in vivo* biochemical pathways vary between species and may mislead the therapist be they doctor or vet, and there seems little point in applying *in vitro* pharmacology to the tissues of other species when it is the healthy, aged or diseased human brain we want to understand. Behavioural studies in the laboratory exclude all the stimulants that make up the world of the wild animal. They are 'nature blind' and the constraints of training suppress the ways the animal might behave to survive in real life [83 rev]. Behaviour is influenced by numerous factors : handling stress, motivation to gather information and the conflicting need to avoid predators, challenge to sexual dominance and changes in hormonal levels. Lesion studies give no hint of mechanisms. Genetic knock-outs ie. molecular lesions take no account of the way in which a developing organism will adapt [84]. Recombinant techniques assume the gene works in isolation from the genetic network and ignores the determining influence of cell, organism and environment on genetic expression [85]. Such studies are (a) unethical and (b) ill-conceived science since their techniques disrupt homoeostatic readjustments, the very mechanisms we need to study, and stimulate responses to genetic insult such as gene silencing and somaclonal variationwith their unpredictable physiological changes [86].

We need more exploration of non-intrusive techniques and instrumentation for gathering information, particularly diagnoses and therapies that utilise the electro-magnetic properties of the *organism.* At present attention is focussed on those of the molecule or individual cell. *In vivo* spectrometric imaging techniques can follow hippocampal and amygdaloidal degeneration [26]. In *vitro* optical imaging of Ca^{2+} dynamics allow studies of cellular excitation [87]; infra red differential interference contrast video microscopy is now so far advanced it enables study of the electrical activity of individual dendrites [88].. Computer simulations and molecular pharmacology throw light on neural circuitry and the factors controlling gene expression [84]. It could be said that animal experimentation is a bar to progress, since if it were discontinued alternative approaches would have to be found and greater attention paid to patient reporting and clinician diagnosis, to the aetiology of an illness and to the aetiology of *health*.

It is strange that pharmacologists have failed to realise we already have a 200-year old pharmacological technique that is safe and controllable, where healthy researchers and volunteers can minutely observe the physical and mental responses to a pharmacological agent. It is known as a homoeopathic proving [89] in which the prover takes low cumulative doses of the test substance and records the unfolding of its effects in those organs of the body where it exerts a cellular influence. Throughout the 19th and early 20th centuries, dedicated provers documented the response to sublethal doses of known toxic substances, and to mineral salts and herbs. Biochemical activators such as cortisone, pepsin and neurotransmitters have also been proved or tested. *In vitro* provings of cell signallers and toxins with cryptic long-term effects will be the next step. One does not have to have a view on the efficacy of homoeopathic treatments to use the provings ie. cumulative challenges, reversible on stopping the dose. These provide the clinician with a holistic analysis that reductionist techniques cannot emulate. The prover however should be healthy. The pathways of an established disease will be down-regulated and can only be diagnosed *in vitro.*

The term "toxicant induced loss of tolerance" (TILT) [81 rev p289-296] has been proposed as more meaningful than MCS in as much as it identifies a toxic origin and recognises a loss of tolerance not only to specific chemicals but also to specific foods. Miller postulates that toxicant induced loss of tolerance characterises a distinct class of disease. In addition to microbial-induced disease and carcinogen-induced disease, we now have disorders induced by novel toxic pollutants and their combinations [90] : asthma, ME, MCS, Gulf War Syndrome, oestrogen mimic reproductive abnormalities etc.

In common with microbes and carcinogens, a toxic agent may attack different organs. Like them, biomarkers are difficult to find. Like them avoidance is the best protection until the pathways are understood [81]. If toxicants comprise a new class of disease, toxicology must revolutionise itself and extend its domain from the study of acute effects to a study of the chronic destructive malfunction that the spent poison, like some radioactive particle leaves in its wake.

13. Conclusion

The medical profession has failed hundreds of farmers, agricultural and other workers, Gulf War veterans and in all probability ME children, by claiming their overt and self-reported symptoms were all in the-mind, Had they insisted on the poisonous connection, it was in their power to have organophosphates banned. The licencing bodies failed them by declaring OP pesticides as safe and denying the emerging evidence of chronic toxicity .MAFF and the Department of Health failed them by wrongly advising they would be protected by the recommended clothing and denying that inhalation was the major danger. Governments failed them by demanding compulsory usage and then denying the problem existed, or if it did that nothing could be done until the link was scientifically proven. No doubt the regulatory and advisory bodies will not move from their position for fear of the huge sums of compensation the Courts might demand of them. The manufacturers are doubly apprehensive: of loss of the billion dollar profits earned by organophosphates and of the litigation brought by all of those around the globe their products have harmed.

OPs must be banned and removed from the environment, not only of the rich but of the third world poor. That leaves unresolved in UK the problem of admission of culpability. Maybe the only way out of the impasse is a moratorium. The Government on behalf of its implicated agencies should admit culpability and provide generous compensation. But what of the chemical companies' culpability? They will never pay reparations for the deaths of hundreds of thousands in developing countries where OPs are sprayed with impunity. Just as they will never pay reparations for the damage done to us and our inheritance, with every land and sea creature and its offspring now carrying a burden of chlorinated hydrocarbons and polychlorinated biphenyls (PCBs). The thousands of noxious products must be taken off the market and irresponsible technology outlawed.

There must be a new 'culture of research' with restoration of publicly funded institutes and universities and researchers' grants and careers safeguarded from commercial pressures. The public and sovereign countries must not be bullied by global corporations and the World Trade Organisation (WTO) into accepting novel products designed to 'save the world' : products now foisted on us by the same global companies that gave us OPs. Concern for the earth and its biodiversity, care for health and the protection of livelihoods must override all other considerations in UK. EU and international law. The guarantee of an adequate world food supply lies in an ecologically sound pest control and an agriculture where nature is the instructor not the victim.

Abbreviations. ACh: acetylcholine, AChE: acetylcholinesterase, ACTH: corticotrophin, ATP: adenosine triphosphate, ChAt: choline acetyl transferase, COPIND: chronic OP-induced neuropsychiatric disorder, CFS: chronic fatigue syndrome, CRH : corticotrophin releasing hormone, CTZ: chemoreceptor trigger zone, EAA: excitatory amino acid, GABA: gamma-amino butyric acid, HPA: hippocampal-pituitary-adrenal stress axis, LTP: long-tenn potentiation, MCS: multiple chemical sensitivity, ME: myalgic encephalitis, NMDA: N-methyl-D-aspartate, OP: organophosphate, POMC: prepro-opiomelanocortin, PTH: parathyroid hormone, PVN : paraventricular nucleus, -R : receptor.

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