DOUBLE BLIND PLACEBO-CONTROLLED CLINICAL TRIAL OF A FOOD SUPPLEMENT CONTAINING PLANT-DERIVED MELATONIN ("ASPHALIA") CLAIMED TO IMPROVE SLEEP IN HEALTHY ADULTS.

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Abstract:

We report here the results of a placebo-controlled double blind trial of a food supplement ("Asphalia") administered orally in powder format. Some 22 candidates (14 female, 8 male) who had signed Informed Consent letters were entered into the trial between October 2004 and March 2005. The Consent Letter asked if the applicant was affected by gluten, cereal products or colourants (a reason for exclusion). Candidates were all healthy adults living in the UK who claimed to be affected by exposure to ambient electromagnetic fields and radiation ("EMF"), and aged between 32 years and 58 years.

The participants matched as far as possible for age and sex were randomly given either a green flour-based powder (placebo) or a sample of the food supplement (Asphalia: "the sample") to be taken just prior to bedtime in water for two weeks, and asked to complete a three part questionnaire. After this period the sample/placebo was substituted for its converse, for ingestion during the next two weeks. The first part was to be completed at commencement and asked *inter alia* for a description of exposure levels to EMF, the second part was to be completed 2 weeks after the start of the course, to reflect the effects of the sample/placebo. The placebo/sample was then changed for its converse. Part 3 was for completion after a further 2 weeks at the end of the trial period, to reflect the effects of its converse. The latter two questionnaires requested a subjective 8-point evaluation of changes in ten specific wellness-related parameters. (Questionnaire forms are shown in Appendix A).

Completed questionnaires were obtained from 16 participants (72.7 percent). The responses were coded and the sleep data extracted before codes were broken to perform statistical analysis (see Table 1 below). None of the candidates reported any significant improvement from placebo ingestion, but whilst taking the placebo one (female) reported a significant deterioration in sleeping pattern, though this candidate turned out to have a history of psychiatric disturbance, and would have been excluded earlier were it known prior to the trial. Of those taking the sample, five (all female) reported moderate sleep improvement, and a higher level of alertness following the course, together with lowered emotional lability ("feeling calmer"). One candidate (female) while taking the sample reported a slight worsening of sleep disturbance (though she also reported moderately lowered nausea). Other effects reported by those taking the sample include improved concentration and lessened memory loss. Two candidates reported slightly lower depression and another reported "feeling better", but one candidate reported slightly increased depression.

We conclude there are distinct indications that ingestion of Asphalia significantly improves sleep without any observed adverse side effects. The minor beneficial effects on depression are difficult to interpret, and will be investigated separately. A further study, stratified by a wider age span, will investigate effects on the elderly, whose melatonin levels diminish with age.

INTRODUCTION

Asphalia is a melatonin-containing food supplement derived entirely from the dried and powdered leaves of gramineous species (wheatgrass, oats, barley, fescue).

"Since the dawn of mankind grasses have provided us with our staple diet and many other needs". These are the opening lines of the Royal Horticultural Society's recent book on grasses (Grounds, 2005). As long ago as the third century BC Theophrastus (c. 370-287 BC) realised that grass seeds germinate with a simple seed leaf (monocotyledon), while those of most other plants germinate with two seed leaves (dicotyledon). This was the beginning of the classification system. Little further progress was made until 1704 when John Ray published a key separating grasses from sedges. The great Carl Linnaeus (1707-1778) finally brought order to the plant world with his sexual system of classification recognising grasses as a distinct group, but it was Bernard de Jussieu (1699-1777) who, in his more natural system of classification gave grasses the status of a family, which he named ***Gramineae***. Modern usage however, requires that plant families take the name of the first genus, and since there is no genus Gramineum the family is now called ***Poaceae***, from the genus Poa.

Historically the first grasses were grown for their grains, providing the staple diets of virtually every society: wheats, ryes, and barleys in Europe, maize in the Americas, millets in Africa, and rice in Asia. Grounds (2005) points out that more plants on earth are grasses than any other kind. In the old Testament God commands mankind to eat grass: *"And ye shall eat the herb of the field"(Genesis, 3.18).*

The nutraceutical value of grasses has an equally long antiquity: a few grasses (e.g. Job's Tears (*Coix lacrima-Job*) were grown in monastery gardens and *inter alia* used for making rosaries from their hard grains, and several others are mentioned in old religious tracts and herbals. For example Gerard in his *Herball* (1597) included couch grass which he said was useful for healing green wounds, and meadow grass, which apparently has the capacity to *"glew and consolidate together new and bleeding wounds*", while the roots of the common reed could be used to draw forth thorns and splinters.

Recent studies of plants reveal that gramineous species contain more melatonin than most fruits and vegetables. The discovery of melatonin as a powerful antioxidant has been comparatively recent, being first isolated by Aaron Lerner only in 1958, who named it from its ability to bleach frog skin (Lerner, Case et al., 1958). For some decades it was thought that in the animal kingdom this indoleamine was synthesised only in the pineal gland in the centre of the brain, and though its control of the circadian rhythms and of breeding cycles was quickly recognised, as well as the diminishing level with age (Sack, Lewy et al., 1987). Only in the last decade has it been realised that melatonin is also synthesised in several other organs including the cornea and the gut, and is also sequestered or synthesised by white blood cells, which when subjected to mitogenic challenge release it into the extracellular fluid at a dose nearly five times the normal physiological level (Carrillo-Vico, Calvi et al., 2004).

Scientific interest in melatonin during recent years has been intense, with many studies establishing its oncostatic, anti-ageing, immune system enhancing and sleep improvement efficacy, and concomitantly its almost complete absence of adverse side effects. No LD50 has ever been established for melatonin: its comparatively small molecular mass and its amphiphilic nature ensures its penetration into any part of the cell, making it far more powerful as a free radical scavenger than any vitamin.

Only a decade ago was it first discovered that melatonin is also present in the seeds, leaves and roots of plants, particularly in graminaceous species, and that plant melatonin is bioavailable to the animal kingdom. A 1995 study reported that among plants the graminaceous species *Festuca arundinacea*

contained more melatonin than any other plant (Hattori, Migitaka et al., 1995). It is this plant which forms the basic ingredient of Asphalia. Though other studies and reviews have appeared since, none have reported levels as high as those found in *F. arundinacea*. Studies at MIT concomitantly reported that pharmacological doses of melatonin (i.e. in the milligram range) were not as effective as physiological doses (i.e. in the micro/picogram range) (Dollins, Zhdanova et al 1994; Zhdanova, Wurtman et al, 1995)).

Accordingly the rationale behind the unique development of Asphalia by this laboratory was that the natural plant-derived product is arguably more effective than the synthetic melatonin produced mainly as a by-product of chocolate manufacture, and when delivered at physiological doses would also prove to be more effective over time.

Health effects of melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) is a hormone and antioxidant which in mammals is largely synthesised from serotonin in the parenchymal cells of the pineal gland, but has been found in other tissues (Huether 1993. Carrillo-Vico 2004). The molecule was first isolated from bovine pineal glands by Aaron Lerner and co-workers (Lerner, Case et al., 1958).
Melatonin has been found in almost all vertebrates tested (Reiter 1991). In vertebrate subjects melatonin is secreted in a daily pattern peaking during darkness hours and subsiding during the hours of daylight. This circadian rhythm led scientists to explore the possibility of a link between melatonin levels and physiological processes such as sleep. Melatonin when administered to human subjects has been shown to modulate circadian rhythms and as a result is useful therapeutically as a treatment for jet lag and other sleep disorders (Arendt, Aldhouse et al., 1986). Several other physiological functions of melatonin have been reported including regulation of reproductive cycles, an anti-aging role, immunoresponsiveness and signal transduction of darkness (Reiter 1991).

Melatonin has been shown to have potent antioxidant action (Tan et al. 2000; Reiter, Tan et al. 2000; Reiter, Manchester et al. 2000) and free radical scavenging properties (Tan, Reiter et al 1993; Reiter, Tan et al. 2000). It has been shown to detoxify hydroxyl radicals, hydrogen peroxide, peroxynitrite anion, nitric oxide and hypochlorous acid (Reiter and Tan 2002). This discovery supported the idea that melatonin may be instrumental in the prevention, cure or control of free radical associated diseases such as Parkinson's and Alzheimer's (Srinivasan 2002). It also provides some support for the possibility that electromagnetic fields may adversely affect melatonin synthesis.

Recent research suggests that melatonin also has a place in the diagnosis (Coudert, B 2002) and treatment of some cancers (Bartsch, Bartsch et al 2002; Vijayalaxmi, Thomas et al 2002; Blask, Sauer et al 2002), with a definite link between physiological melatonin levels and cancer incidence. Suppression of the normal light/dark melatonin cycle by light during darkness hours can lead to an increase in the progression of cancerous tissue (Blask, Dauchy et al 2002; Reiter, 2002; Sanchez-Barcelo, Cos et al 2003).Cancer patients tend to have a lower secretion of melatonin compared to healthy subjects (Kos-Kudla, Ostrowska et al 2002). Melatonin has been shown to have an inhibitory effect on the growth of endometrial (Kobayashi, Itoh et al 2003), breast (Dillon, Easley et al 2002; Anisimov 2003; Bizzarri, Cucina et al 2003) and prostate (Shiu, Law et al 2003) cancer tumours.

It seems that not only can melatonin inhibit the growth of cancer tumours but has been shown by Lissoni et al. to reduce the toxic effects of chemotherapies (Lissoni, Chilelli et al 2003). This trial showed that both tumour regression and 5-year survival rates were higher in those concomitantly treated with melatonin. No patient treated with chemotherapy alone was alive after 2 years; in contrast 5-year survival was achieved in 6% of patients treated with melatonin.

Recent research has shown that children receiving chemotherapy for acute lymphoblastic leukaemia (ALL) have a lowered blood antioxidant status compared to that before the commencement of treatment, making them more prone to complications from oxidative stress (Kennedy, Ladas et al., 2005). In view its beneficial action in situations of oxidative stress (Maestroni, 1988) there may be a place for melatonin therapy administration alongside ALL treatments.

Notwithstanding that melatonin has been reported to have a number of beneficial medical effects for human subjects, *inter alia* with primary and secondary sleep disorders, this study is confined to the sleep improvement reported in normal healthy subjects by a number of recent studies as a consequence of melatonin administration. Accordingly the effects of melatonin on primary and secondary sleeping disorders are not addressed by this study.

Brief literature review of clinical trials (melatonin and sleep)

Scientific literature on melatonin has quickly grown to extend to more than ten thousand peer reviewed studies, so great is scientific interest in this indoleamine. Confining attention to clinical trials on sleep in normal healthy subjects following melatonin administration reveals at least 22 clinical trials in the last 20 years, of which nearly all report a positive effect of melatonin on various sleep parameters. In all cases however, only synthetically-produced melatonin was trialled, so our study is the first to report biological effects of melatonin derived from natural plant materials.

Table 1: Clinical trials of melatonin and sleep patterns in normal healthy subjects

Author(s)	Year	**Subjects**	Outcome	Conclusions
James, Mendelson et al	1987	10	1 mg-5mg dose: Increase in REM latency, but no other REM-related effects.	"Could not find any other change in onset or duration of sleep".
James, Sack et al	1990	10	1 mg dose: sig. ↑ in REM latency.	"Less sleep reported., but overall subjective (sleep) quality was improved".
Ferini-Strambi, Zucchoni et al	1993	6	100 gm dose: ameliorated sleep quality	"Combined melatonin and low benzodiazepine doses could avoid the residual benzodiazepine effects"
Oldani, Ferini-Strambi et al	1994	6	Sig.↑ advance in sleep onset & awakening hour	"Objectively confirms previous data obtained by a sleep-wake diary".
Dollins, Zhdanova et al	1994	20	**0.1-10mg dose:** Sig. ↑ in sleep duration, sig. . decrease in sleep onset latency	"These data indicate that orally administered melatonin can be a highly potent hypnotic agent"

Zhdanova, Wurtman et al	1995	6	**Oral 0.3 or 3mg** dose decreased sleep onset latency	"These data provide new evidence that …exogenous melatonin may be useful in treating insomnia"
Allenburrow, Cowen et al	1996	15	Oral 1mg dose: sig. ↑ in actual sleep time etc.	"These data are consistent with the hypothesis that low dose melatonin has hypnotic effects on humans"
Zhdanova et al	1996	12	**0.3-1 mg dose**: sig. decrease in sleep latency	Young healthy adults: 0.3 mg dose elevated serum concentration to 113pg/ml
Cajochen, Krauchi et al	1996	8	Oral 5 mg dose: subjective sleepiness in 40-90 mins	"Sig. correlation between subjective sleepiness and salivary melatonin levels"
Garfinkel, Laudon et al	1997	21	Oral 2mg dose: sig. ↑ in sleep efficiency etc	"Melatonin replacement therapy can sig. increase sleep quality in the elderly and the beneficial effects are ↑ in the presence of benzodiapines"
Nagtegaal, Kerkhof et al	1998	30	Sig.↑ in sleep onset. Sig. decrease in sleep latency	"DSPS patients felt sig. more refreshed in the morning"
Cajochen, Krauchi et al	1998	10	5 mg dose: shortens sleep latency	Adversely affected by 3hrs bright light (5000 lux)
Okawa, Uchiyama et al	1998	11	↑ in 6/11 patients	"Timing and dose should be further investigated"
Matsumoto M	1999	6	↑ increase in total sleep time	"Melatonin at 10 am had direct hypnotic effects on diurnal sleep"
Luboshitzky, Levi et al	2000	6	6 mg dose: no chronic effect on secretory patterns	"Mean nocturnal LH, FSH, and testosterone levels did not change during 1 mth treatment period".
Pires, Benedito-Silva et al	2001	6	Sig. ↑ effects of **0.3mg dose**	"Low dose may exert immediate sleep inducing effects"
Satomura, Sakamoto et al	2001	7	1,3, 6mg dose: sig ↑ in total sleep time and efficiency	"Exogenous melatonin had dose dependent hypnotic action on daytime sleep"
Paul, Brown et al	2001	13	10mg dose: 370 hrs sleep vs. 339 hrs (placebo)	"Both zopicione and melatonin improved sleep relative to placebo".
Smits, Nagtegaal et al	2001	40	1-5mg dose:	Children 6-12 with chronic

			sig more effective in advancing sleep onset	sleep disorder: no side effects noted.
Sharkey & Eastman	2002	32	**0.5 mg dose**: Phase advance 3.0 hrs vs. only 1.7 hrs (placebo)	"Melatonin could be used to promote adaptation to night work and jet travel".
Kunz, Mahlberg et al.,	2004	14	3mg dose: sig. increase in REM sleep	"Exogenous melatonin seems to normalize circadian variation".
Mundey, Benloucif et al	2005	13	Sig advance in circadian clock	"0.3 and 3 mg doses both advanced the circadian phase"

This table is instructive in that a quarter of these studies report virtually an immediate significant effect on sleep at doses at or below 500 μgrams, and 300μg doses can induce serum melatonin concentrations in excess of 100pg/ml. This is important, since although some animal studies have found effects after ingestion of melatonin-rich plants, levels of the indole reported in plants is several orders of magnitude below doses administered at pharmacological levels in the clinical trials listed above. This is illustrated in the brief review of the plant melatonin literature below.

Brief literature review relating to plant-derived melatonin

In response to the reported health benefits of melatonin there are many synthetically produced melatonin tablets on the market. These are taken by a wide range of people including sufferers of insomnia and jet lag as well as those who simply want to preserve their good health. Some people cannot freely obtain supply since in some countries (e.g. UK) melatonin is a prescription-only medicine (POM). In addition there are those people who do not wish to take synthetic medicines but would like the ability to supplement their endogenous melatonin.

Epidemiological studies indicate that consumption of fruit and vegetables can protect against a variety of diseases (Doll 1990; Dragsted, Strube et al 1993). The chemical components responsible for these preventative properties are thought to be antioxidants such as vitamins C and E, β-carotene and flavonoids. However, some plant tissues contain melatonin and therefore the consumption of such tissues could alter blood melatonin levels and offer antioxidant protection in addition to endogenously produced melatonin (Hattori, Migitaka et al 1995).

The discovery of melatonin in algae (Poeggeler and Hardeland, 1994) led to speculation that melatonin may be found in a wider range of plant tissues. Melatonin in higher plants was first reported by Hattori et al. (Hattori, Migitaka et al 1995). Hattori determined the melatonin level of twenty four edible plants; selected examples are listed in Table 2. The same paper reported that plasma melatonin levels in birds increase after feeding plant products which are rich in melatonin it also showed that plant derived melatonin binds to melatonin receptors in rabbit brain. These findings are important as they indicate that vertebrates can supplement their endogenous melatonin according to the plant material they consume.

More recently Badria has published data on the levels of the indoles tryptamine, melatonin and serotonin in what he describes as Egyptian food and medicinal plants (Badria 2002). In total nineteen

plants were tested, many of the plants were the same as tested by Hattori, a selection of these are listed in Table 2 for comparison.
Analysis of melatonin in seeds has been investigated (Manchester, Reiter et al 2000). Melatonin was found in all fifteen seeds sampled, the highest levels were found in mustard seeds. It has been suggested (Manchester, Reiter et al 2000) that the high levels of melatonin offer protection to the germ tissue of the seed from environmental factors such as UV light, drought, extremes of temperature and chemical pollution.

Table 2: Melatonin levels detected in selected edible plants.

Plant	Melatonin ng/g plant tissue				
	Hattori et al (RIA)	**Badria (GC-MS)**	**Reiter/Tan (HPLC)**	**Dubbels et al (HPLC-MS)**	**Manchester (RIA)**
Banana		0.655		1	
Barley	0.378	0.873			
Cabbage	0.107	0.309			
Carrot	0.055	0.494			
Cherry (Balaton)			2		
Cherry (Montmorency)			15		
Corn	1.366	1.878			
Cucumber	0.0246	0.592			
Festuca	5.288				
Ginger	0.538	1.423			
Oats	1.796				
Onion	0.032	0.299			
Pineapple	0.036	0.278			
Rice	1.006	1.498			
Strawberry	0.0124	0.136			
Tomato	0.0322	0.302		2-8	
White mustard seed					189
Black mustard seed					123
Wolfberry					103

Table 3: Melatonin levels in Chinese and alpine medicinal plants.

Plant	**Melatonin ng/g plant tissue**			
	Reiter/Tan	**Zhang (HPLC-FD)**	**Tettamanti**	**Murch**
Huang qin	7110	178		2190
Chantui		3771		
St Johns Wort	4390[a]/1750[b]		10.901	2450[c]/1920[d]
Yarrow			43.154	
Marsh-Mallow			22.737	
Lemon verbena			22.222[e]/16.631[f]	
Balm mint			15.924	
Peppermint			19.521	
Sage			29.343	
Thyme			13.210	
Scullcap				1610
Feverfew				1690

a flower
b leaf
c flower
d leaves
e young plant
f dried leaves

The highest melatonin levels have been measured in plants traditionally used for medicinal purposes. Chinese medicinal herbs have been used for centuries to alleviate or cure many diseases. Zhang et al (Zhang, Chen et al 2003) analysed melatonin levels in 108 Chinese medicinal herbs. Over half of the herbs tested contained melatonin levels in excess of 10ng/gm, some herbs contained levels in the µg/gm range. Of particular interest was that many of the herbs containing the highest melatonin levels are traditionally used to treat diseases associated with free radicals.

Alpine medicinal plants have been found to contain high levels of melatonin (Tettamanti, Cerabolini et al. 2000). It has been suggested that melatonin may be required by these plants to allow them to survive at high altitude. UV and ozone are at higher levels in these environments, and it is already known that ozone in high concentrations decreases plant height and causes foliar damage.

It is apparent from the data that there are marked differences in the mean amounts of melatonin between plants of the same species. There could be a number of reasons for this. Firstly, the methods of quantification differ amongst research groups. The common methods of quantitation are radioimmunoassay (RIA), high performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GC-MS). RIA, HPLC and GC-MS are capable of detecting daytime (10pg/ml) and night-time (30-120pg/ml) melatonin in plasma/serum (Saunders, Chaturvedi et al., 1998)

Radio immunoassay (RIA) is a favoured method of melatonin determination in biological fluids due to its simplicity and sensitivity. Wide usage of RIA can also be attributed to the wide availability of commercial melatonin kits. Levels of melatonin in plant tissue were determined by Hattori et al. using RIA (Hattori, Migitaka, et al., 1995). The problem with RIA is lack of specificity of the antibody causing erroneous results due to cross reaction with other closely related indolic analogues e.g serotonin, tryptophan, 5 hydroxy-indoleacetic acid. Extraction of melatonin from the sample in question followed by the RIA goes some way towards alleviating this problem.

HPLC has often been coupled with fluorescence detection. It is possible that interfering species may give erroneous results in the fluorescence.

GC-MS techniques for the determination of melatonin are used to a lesser extent mainly due to their being difficult to fully automate compared with bioassays. However, GC-MS is far more specific, especially in view of today's mass spectrometry technology such as selected ion monitoring and MS^n techniques, which ensure accurate identification and quantification of samples. RIA analyses on plants have been verified by GC-MS of the residual extracts (Dubbels, Reiter et al 1995). Measurement of melatonin in Egyptian food plants (Badria 2002) used GC-MS methods.

Secondly the environment of the growing plant may have an effect on the melatonin levels. The main factor which may differ is the time of year at which the plant was harvested and the resulting conditions it was grown under. For example plants grown under glass would experience different conditions compared to those grown outdoors, similarly plants grown in different climates would be subject to different environmental influences.

In short, from the published data it can be seen that melatonin is found in a wide range of plants at varying concentrations. The variation of amounts measured in plants of the same species could be due to either differing growing environments or use of different analytical techniques for detecting melatonin. The highest amounts of melatonin have been observed in grasses and those plants traditionally used for medicinal purposes, which could help explain their therapeutic action. Although the function of melatonin in plants has not been established it seems that its antioxidant properties may have a protective role on vulnerable tissues.

The discovery that the consumption of high melatonin foodstuff raises plasma melatonin and binds to brain receptors of vertebrates opens a new avenue of investigation into whether dietary melatonin is more or less effective than synthetic tablets, and it could be that supplementing endogenously synthesised melatonin with high melatonin foodstuffs is more desirable than recourse to synthetic tablets. This consideration lies behind the rationale for present trial.

METHOD AND MATERIALS

The study protocol is of randomised double blind placebo-controlled design.

The safety of short term melatonin administration was confirmed by reference to a recent meta-analysis which reported that 17 randomised controlled trials showed no adverse effects of melatonin with short term use of three months or less (Buscemi, Vandermeer et al., 2005). The separate ingredients of Asphalia are all well established as non-toxic traditional cereal-related foods or grazing forage for livestock. The variety of fescue seed supplied was of an endophyte-free strain (Barenbrug UK) and this was cultured for two years in organic soil. Only the air-dried leaf was used to mill the final powder, and at slow speed to avoid denaturing the protein content of the plant. When ground the powder was maintained at 4 degrees Centigrade until posted to the subject for administration.

Candidates were recruited into the study via a heterogeneous population spread across the UK who identified themselves to a website (www.mastsanity.co.uk) advising the general public on putative health effects from chronic exposure to radiations emitted by cellphone masts. This website was contacted seeking volunteers for testing a "radioprotective food supplement". Candidates were provided with an informed consent form describing it as a meadowgrass powder similar to barley and wheatgrass powders. No mention was made that the aim of the study was to assess sleep improvement effects, and the questionnaire buried the sleep-related questions within a list of ten unrelated symptoms or minor ailments commonly associated with EMF exposure in epidemiological studies of radiation sickness (Silverman, 1980).

Blindedness was obtained by ensuring that the subjects were unaware whether they were receiving a placebo or a sample, and the analysis of questionnaires was carried out by one of us (RWC) without knowing whether the response related to placebo or sample. No reports indicated that the subjects were able to unblind the study through their own immediate subjective responses to soporific effects, as some studies have reported with much higher melatonin doses. The placebo was produced from a gluten-free green food colourant (Supercook, Sherburn in Ermet, Leeds UK) mixed with domestic baking flour to a shade indistinguishable from the sample.

Successful candidates were asked to take one dose of the sample/placebo (one level teaspoon each evening at bedtime, equivalent to 0.8 gm of dried powder), allocated randomly between subjects so that though overall they underwent one fortnight period of taking the sample and one fortnight taking the placebo, the order was randomly allocated. In considering whether to include a washout period we followed other trials which had not done so on the basis that melatonin synthesis/administration and excretion is of short term duration (a few hours), so the two test periods were contiguous.

Calculation of melatonin content of dose, derived from our own spectrophotometric analysis (ATI Unicam UV2), confirmed by GC/MS/MS (Finnigan Polaris Q, Thermo Electron) and supported by an independent study (Hattori et al 1995) indicated that each dose contained approx 1.12 □grams of melatonin, hence is two orders of magnitude lower than the lowest dose administered in previously reported clinical trials. This issue is addressed in the discussion.

Questionnaires were in three parts (see Appendix 1), the first to be completed before starting the four week course, the second two weeks into the course and the third at the end of the second two week period. Questionnaire 1 captured location, age, and sex-related data, and brief information on EMF exposure levels, medical history, and the putative radiation derived symptoms complained of. Questionnaires 2 and 3 collected subjective details of changes in a list of ten symptoms including sleep disturbance noted after taking the sample/placebo for two weeks, and asked to record any lapses in the administration regime. Appended to Questionnaire 3 was a further overall question asking for any change noted in health condition on a seven point scale since starting the course, from "considerably worse" to "considerably better", passing through "no difference". It also asked for details of any major lifestyle changes during the course.

RESULTS

Of the 22 candidates who completed Consent Forms, completed questionnaires were obtained either postally or by telephone interview from 16 (72.7 percent). Of these 8 were female and 8 were male. The average age was 48.4 (males 46.2, females 52.6) and the range of ages was between 32 and 58. 3 subjects did not take the entire course but missed upto 3 days administration. One female subject completed the questionnaire but not the course, having suffered a headache on day 2 (she attributed this to operation of a new 3G mast in the vicinity). 5 subjects reported an improvement in sleep and 2 subjects reported a slight improvement. One subject also reported feeling calmer. 7 subjects reported no change or noticeable effect.

Scoring was on a ten point scale, from -5 to +5, representing a range from "Considerably worse" to "Considerably better" sleep during the two active study periods.

The nul hypothesis predicts that the mean overall score would be +/-0.00, i.e. that there would be no improvement or deterioration in sleep parameters from the administration of either the active sample or the placebo. The results showed, however, that the mean overall score for the active sample was calculated to be +1.81 (SD -2.83), (with +5 representing considerably better sleep, 0.00 representing no change, and -5 representing considerably worse), and the placebo overall mean score was 0.13 (SD -0.62). Due to the scoring system deployed, which was conservative to allow for the subjective nature of responses, the consequent SDs clearly showed a wide spread.

Estimated effect size (Yi) was calculated according to the method of Brzezninski et al (2005), where $Y_i = Xbar_a - Xbar_p$ these being the mean responses on active and placebo respectively. This produces Yi = 1.68 and an SEM(Yi) of 2.62.

No candidate reported deterioration in health condition at the conclusion of the trial period, but seven subjects reported improvement. Follow-up at six months established that this lack of deterioration had continued and that several of the subjects were now regular satisfied users of Asphalia. We interpret the mean positive overall score to indicate a moderately successful sleep improvement trend overall during the active period compared with placebo (the placebo mean overall score was 0.13).

Individual overall scores are shown in Table 4. Applying a test of matched pairs (see Colton, *Statistics in Medicine*, 1974, p135) indicates that the effect is significant ($p = <0.01$).

Table 4: Summary of individual trial subjects' data.

Subject	Sex	Age	Outcome			Comments	Location	Difference
			active	placebo	difference			
PB	M	44	0	0	0	Missed 3 days	Stroud	None
RB	F	na	5	0	5	Slept whole night	W Sussex	Calmer
IC	F	47	0	-1	-1	Missed 2 days	Stroud	None
AC	M	na	0	0	0		Barry, Glam	None
SF	F	54	4	0	4	Parkinson's controlled	Manchester	Improved
RG	F	44	5	1	4	Much more sleep	Haverfordwest	Improved
LI	F	na	5	0	5	Slept right through	Sutton Coldfield	Improved
KS	M	32	2	2	0	Stress during trial	Ormskirk, Lancs	Slightly Improved
JM	F	50	4	0	4	Slept well	London	Improved
RM	M	54	3	0	3	Felt fresher	London	Slightly Improved
CM	F	44	3	0	3	Much better sleep some days	Redditch	Improved
HN	M	na	0	0	0		Glastonbury	None
BP	M	na	0	0	0		Barmouth	None
PW	M	na	0	0	0		Dursley. Glos	None
SW	F	58	-2	0	-2	Headache on day 2	Wolverhampton	Didnt complete trial
PW	M	57	0	0	0	Missed 1 dose	Wolverhampton	None
	Totals		29	2	31			
	Means:	48.4	1.81	0.13	1.56			
		SD:	-2.32	-0.62	-2.34			

* score on a ten point scale of the active sample effects relating to subjectively reported sleep improvement (see above for definitions).

(sum of squares: 121; $(sum)^2/n$: 60.06; sum of squares about the mean: 60.94; s^2d: 4.06)

Applying the t test of significance for paired samples

$$\frac{1.94 - 0}{0.5}$$

$$t_{15} = \frac{\overline{d - \overline{d0}}}{\sqrt{s^2 d / n}} = \quad = 3.88$$

With 15 degrees of freedom t is more than 2.947, and therefore significant (p=<0.01).

DISCUSSION

When comparing the active sample effects these results, though based on self-reported subjective data, suggest that an otherwise distinct improvement in sleeping pattern as a result of melatonin-rich plant administration may be adversely affected if dose is discontinued for some days. The cessation of beneficial effect when melatonin treatment was stopped was also reported (Dahlitz et al, 1991). Several studies have reported inhibition of melatonin by electric fields, but whether the absence of improvement is due to any chronic EMF exposure of this particular group is not clear. A disadvantage was that some subjects wrote copiously of their trial experience rather than complete the questionnaire rigorously and fully, and this might have influenced the interpretation of scoring. If the three subjects who missed days administration are excluded, the overall score rises moderately to +2.23 in favour of a beneficial effect on sleep (placebo +0.13).

The question of very low dosage

Addressing the question of the very low dose (some two orders of magnitude below all other studies) it must be surprising to see that such low dose (at physiological rather than pharmacological levels) can nevertheless have a usefully positive effect on sleep quality, and brings into question the possibly confounding effect of placebo: any administration offered ostensibly as a sleeping aid might benefit from the placebo effect. In this study however the subjects were unaware of the true aim of the study, and the other nine masking parameters offered for response (e.g. depression, memory loss, nose bleed, emotional lability, lack of concentration) did not report a similar overall level of improvement.

Some studies of elderly subjects given low (100μg) doses also confirm that these translate into increases of serum melatonin concentrations of around 40μg/ml (Shah, Langmuir et al, 1999). In a recent meta-analysis moreover out of 15 studies of healthy subjects 11 reported effects at <500□g concentrations (Brezezinski, Vangel et al., 2005). These authors comment that *"The dose response relationships in 11 of the studies support the existence of a plateau, with maximum effect being achieved at low doses (e.g. 0.3mg) and maintained or* ***diminished*** *at higher doses"*. This finding gives some support to the hypothesis that low dose can be even more effective than higher dose.

Moreover there is also some support from animal studies, in that physiological doses of melatonin-rich feeds are capable of inducing beneficial effects in laboratory, livestock, and domestic animals. For example, a dose of 0.3 μg/g via feed pellets produced 15nM in the serum of chickens, which is more than ten times higher than the nocturnal peak melatonin concentration, within 2 hours (Noddegaard and Kennaway, 1999). Hattori's 1995 groundbreaking paper on melatonin in plants included a study where two-week female chicks were fed on prepared melatonin-rich (3.5ng/gm) and melatonin-poor (<100pg/gm) feedcorn, and found the difference was significantly reflected in their plasma melatonin levels within hours. The results demonstrate that even this extremely low melatonin concentration passes through the gastrointestinal wall and enters the blood, thereby increasing plasma melatonin levels detectably. The previous year a group from MIT had reported similar effects with 100□g doses on humans.

In considering how such a vanishingly small administration of melatonin could have such potent effects (without invoking homeopathic principles) one must remember that the initial target tissue is blood. Oral ingestion of melatonin is detected in the bloodstream very soon

after administration, as indicated above. Moreover other small biomolecules affecting blood can also exert powerful effects with extremely small doses: the anticoagulant heparin for example has no known lower limit to its efficacy, and snakebite envenomation of the Indian King Cobra is lethal to mice at doses of 2.5 µg/20 gm (Saha, Gomes et al, 2006).

Another issue concerns the study population itself. All participants are self-classed as healthy in terms of their physical state, but since they were all from a population which believes that cellphone masts have adversely affected their health, the question arises over the psychological condition of the study sample. To address this issue a review of the literature reporting health conditions among populations vicinal to radio transmitters is needed. Though the topic is controversial there are a continuing majority of studies now being published reporting adverse health effects well below the ICNIRP ("thermal") guidelines, and the possibility that environments vicinal to masts and high voltage powerlines are malign in the long term can no longer be dismissed (see Cherry 2000 for review). For example a recent large UK study reported near-doubled incidence of childhood leukaemia near high voltage powerlines (Draper, Vincent et al., 2005).

The issue of electropollution and sleep disturbance

The topic of adverse health effects from chronic exposure to RF/MW/ELF fields and radiation is highly controversial with large research initiatives completed or in progress without so far any consensus, and opposite positions being taken by equally reputable scientific institutions. Since the study population are taken from those believing that their sleep is being disturbed by RW/MW exposure it is instructive to examine briefly if their might be any scientific basis for their concerns.

The WHO, in formulating its advice regarding EMR, takes into account only properly peer reviewed and published studies, and this is a precept we follow in this text. It is therefore useful at the outset to divide such studies into those reporting biological effects at levels below the guidelines (i.e. at levels generally called "non thermal") and those finding no such effects at those field strengths or power densities.

Some scientists believe that even the published studies show bias depending on the source of funding. This view is well illustrated by Table 5 below summarising the results of some 307 studies:

Table 5: Cell Phone Biological Studies

	Effect	**No Effect**	**Total**
Industry-Funded	27 (29%)	66 (71%)	93 (30%)
Non-Industry-Funded	147 (69%)	67 (31%)	214(70%)
Total	**174 (57%)**	**133 (43%)**	**307**

$X2 = 39.93$ ($p < .001$)

(Source: Prof Henry Lai, University of Washington, Seattle, 3/3/06)

A brief summary of the studies giving rise to concern is presented in Table 6.

Table 6: Studies reporting biological effects of radiofrequency radiation (RFR) at low intensities

(1) Balode (1996)- blood cells from cows from a farm close and in front of a radar showed significantly higher level of severe genetic damage.

(2) Boscol et al. (2001)- RFR from radio transmission stations (0.005 mW/cm2) affects immunological system in women.

(3) Chiang et al. (1989)- young people who lived and worked near radio antennae and radar installations showed deficits in psychological and short-term memory tests.

(4) De Pomerai et al. (2000, 2002) reported an increase in a molecular stress response in cells after exposure to a RFR at a SAR of 0.001 W/kg. This stress response is a basic biological process that is present in almost all animals - including humans.

(5) De Pomerai et al. (2003) RFR damages proteins at 0.015-0.02 W/kg.

(6) D'Inzeo et al. (1988)- very low intensity RFR (0.002 – 0.004 mW/cm2) affects the operation of acetylcholine-related ion-channels in cells. These channels play important roles in physiological and behavioral functions.

(6) Dolk et al. (1997)- a significant increase in adult leukemias was found in residents who lived near the Sutton Coldfield television (TV) and frequency modulation (FM) radio transmitter in England.

(8) Dutta et al. (1989) reported an increase in calcium efflux in cells after exposure to RFR at 0.005 W/kg. Calcium is an important component of normal cellular functions.

(9) Fesenko et al. (1999) reported a change in immunological functions in mice after exposure to RFR at a power density of 0.001 mW/cm2.

(10) Hjollund et al. (1997)- sperm counts of Danish military personnel, who operated mobile ground-to-air missile units that use several RFR emitting radar systems (maximal mean exposure 0.01 mW/cm2), were significantly lower compared to references.

(11) Hocking et al. (1996)- an association was found between increased childhood leukaemia incidence and mortality and proximity to TV towers.

(12) Ivaschuk et al. (1999)- short-term exposure to cellular phone RFR of very low SAR (26 mW/kg) affected a gene related to cancer.

(13) Kolodynski and Kolodynska (1996)- school children who lived in front of a radio station had less developed memory and attention, their reaction time was slower, and their neuromuscular apparatus endurance was decreased.

(14) Kwee et al. (2001)- 20 minutes of cell phone RFR exposure at 0.0021 W/kg increased stress protein in human cells.

(15) Lebedeva et al. (2000)- brain wave activation was observed in human subjects exposed to cellular phone RFR at 0.06 mW/cm2.

(16) Magras and Xenos (1999) reported a decrease in reproductive function in mice exposed to RFR at power densities of 0.000168 - 0.001053 mW/cm2.

(17) Mann et al. (1998)- a transient increase in blood cortisol was observed in human subjects exposed to cellular phone RFR at 0.02 mW/cm2. Cortisol is a hormone involved in stress reaction.

(18) Marinelli et al. (2004)- exposure to 900-MHz RFR at 0.0035 W/kg affected cell's self-defense responses.

(19) Michelozzi et al. (1998)- leukemia mortality within 3.5 km (5,863 inhabitants) near a high power radio-transmitter in a peripheral area of Rome was higher than expected.

(20) Michelozzi et al. (2002)- childhood leukemia higher at a distance up to 6 km from a radio station.

(21) Navakatikian and Tomashevskaya (1994)- RFR at low intensities (0.01 - 0.1 mW/cm2; 0.0027- 0.027 W/kg) induced behavioral and endocrine changes in rats. Decreases in blood concentrations of testosterone and insulin were reported.

(22) Novoselova et al. (1999)-low intensity RFR (0.001 mW/cm2) affects functions of the immune system.

(23) Novoselova et al. (2004)- chronic exposure to RFR (0.001 mW/cm2) decreased tumor growth rate and enhanced survival in mice.

(24) Park et al. (2004) higher mortality rates for all cancers and leukemia in some age groups in the area near the AM radio broadcasting towers.

(25) Persson et al. (1997) reported an increase in the permeability of the blood-brain barrier in mice exposed to RFR at 0.0004 - 0.008 W/kg. The blood-brain barrier envelops the brain and protects it from toxic substances.

(26) Phillips et al. (1998) reported DNA damage in cells exposed to RFR at SAR of 0.0024 - 0.024 W/kg.

(27) Polonga-Moraru et al. (2002) change in membrane of cells in the retina (eye) after exposure to RFR at 15 mW/cm2.

(28) Pyrpasopoulou et al. (2004) exposure to cell phone radiation during early gestation at SAR of 0.0005 W/kg (5 mW/cm2) affected kidney development in rats.

(29) Salford et al. (2003)- nerve cell damage in brain of rats exposed for 2 hrs to GSM signal at 0.02 W/kg.

(30) Santini et al. (2002)- increase in complaint frequencies for tiredness, headache, sleep disturbance, discomfort, irritability, depression, loss of memory, dizziness, libido decrease, in people who lived within 300 m of mobile phone base stations.

(31) Sarimov et al. (2004)- GSM microwaves affect human lymphocyte chromatin similar to stress response at 0.0054 W/kg.

(32) Schwartz et al. (1990)- calcium movement in the heart affected by RFR at SAR of 0.00015 W/kg. Calcium is important in muscle contraction. Changes in calcium can affect heart functions.

(33) Somosy et al. (1991)- RFR at 0.024 W/kg caused molecular and structural changes in cells of mouse embryos.

(34) Stagg et al. (1997)- glioma cells exposed to cellular phone RFR at 0.0059 W/kg showed significant increases in thymidine incorporation, which may be an indication of an increase in cell division.

(35) Stark et al. (1997)- a two- to seven-fold increase of salivary melatonin concentration was observed in dairy cattle exposed to RFR from a radio transmitter antenna.

(36) Tattersall et al. (2001)- low-intensity RFR (0.0016 - 0.0044 W/kg) can modulate the function of a part of the brain called the hippocampus, in the absence of gross thermal effects. The changes in excitability may be consistent with reported behavioral effects of RFR, since the hippocampus is involved in learning and memory.

(37) Vangelova et al. (2002)- operators of satellite station exposed to low dose (0.1127 J/kg) of RFR over a 24-hr shift showed an increased excretion of stress hormones.

(38) Velizarov et al. (1999) showed a decrease in cell proliferation (division) after exposure to RFR of 0.000021 - 0.0021 W/kg.

(39) Veyret et al. (1991)- low intensity RFR at SAR of 0.015 W/kg affects functions of the immune system.

(40) Wolke et al. (1996)- RFR at 0.001W/kg affects calcium concentration in heart muscle cells of guinea pigs.

These published studies are a representative sample of those now emerging. Typical of those causing concern among experts is that of Fiorenzo Marinelli (see ref 18 above) from the University of Bologna (where in 1795 Luigi Galvani first observed that a radiowave caused by a simple small spark at one end of his laboratory could demonstrably activate a frog's leg muscle at the other).

Marinelli underlined the potential danger of cellphone base station radiation by research that showed how radio waves from these stations trigger and promote the growth of cancers. Paradoxically, the study suggests that the radiation makes tumours grow more aggressively after initially killing off cells of all kinds including cancer cells.

Marinelli and his team at the National Research Council in Bologna, Italy, decided to investigate whether radio waves had any effect on leukaemia cells after previous studies indicated that the disease might be more common among mobile phone users. The life cycle of leukaemia cells is well understood, making it relatively easy to spot changes in behaviour.

The team exposed leukaemia cells in the lab to 900 MHz radio waves at a power level of 1 milliwatt, and then looked at the activity of a gene that triggers apoptosis (cell suicide). Many European mobile networks operate at 900 MHz, and power outputs are typically 2 watts, although at times using only one-tenth of this power (which is still well in excess of 1 milliwatt).

After 24 hours of continuous exposure to the radio waves (this is the kind of exposure experienced by people and especially children, living or schooling near to inappropriately sited base stations), the suicide genes were turned on in far more leukaemia cells than in a control population that had not been exposed. What is more, 20 per cent more of all cells among those exposed had died than in the non exposed controls.

But after 48 hours exposure, the apparently lethal effect of the radiation went into reverse. Rather than more cells dying, Marinelli found that a survival mechanism kicked in. Three genes that trigger cells to multiply were upregulated (turned on) in a high proportion of the surviving cells, making them replicate ferociously. The cancer, although beaten back for a brief spell, had become more aggressive.

Marinelli first presented his results in 2002 at the International Workshop on Biological Effects of Electromagnetic Fields in Rhodes. While these results do not show a direct health threat from intermittently used mobile phones, they do show a direct health threat from mobile phone base stations, and they provide fresh evidence that the low level radiation from all such devices using these frequencies and power levels, play an important role in activating genes that can trigger cancers and help cancer cells multiply aggressively.

"We don't know what the effects are on healthy human cells," says Marinelli. "But in leukaemia cells the response is always the same." Marinelli suspects the radiation initially damages DNA, and that this interferes with the cells' biochemical signals in a way that ultimately triggers a 'defensive' mechanism.

This work supports earlier findings by Lai and Singh that RF/MW radiation can cause single and double strand breaks in DNA.

Some scientists offer the misleading notion that because radiation from cellphones does not have enough energy to break chemical bonds, it cannot damage cells. The only way damage could occur, they argue, is if the radio waves heated tissues up (the notorious “cooking” effect relied on by the former NRPB). But much published research (as shown above) including British research earlier in 2002, by molecular toxicologist David de Pomerai at the University of Nottingham, showed that radio waves can cause biological effects that are not due to heating. He found that nematode worms exposed to radio waves showed an increase in fertility - the opposite effect from what would be expected from heating.

The Stewart report in April 2000 funded by the British Government found no evidence of any health risks from mobile phones, but it still recommended that people take a precautionary approach until further evidence emerged. In particular, it suggested children, whose brains are still developing, should not use mobile phones excessively. Some thirty important studies were curiously omitted from that report, however, which may have been the reason for its lukewarm conclusions

"It's a very confused field," claims Colin Blakemore, a physiologist at the University of Oxford and a member of the British National Radiological Protection Board's advisory group on non-ionising radiation.

But de Pomerai at first insisted that a scientific consensus has emerged that non-ionising radiation indirectly damages DNA by affecting its repair system. If the DNA repair mechanism does not work as well as it should, mutations in cells can accumulate, with disastrous consequences. "Cells with unrepaired DNA damage are likely to be far more aggressively cancerous," he concluded at the time. Since then his funding source (and his opinions) have changed, however.

The issue of chronic versus acute effects

Cellphone handset usage is self-evidently both elective and acute: users can themselves choose how long to accept exposure. By contrast, exposure to cellphone mast radiation is chronic (all day and night, continuously) and the exposee has no say in the radiation dose, hence the exposure is non-elective and solely within the gift of the planning authority. This predicates the issue of cumulative exposure, a topic which has already raised concerns in relation to handsets, where several studies have found an elevated incidence of acoustic neuroma among those using the instruments over five or so years.

Therefore the researcher faces another problem in evaluating and setting standards, namely the different time periods used to express the values. The listed field intensities of recommended exposure levels must be compared carefully since they refer to different exposure times As stated above the significant factor is the accumulated exposure over a relative short length of time, but the regulatory guidelines and codes do not give any value for a total accumulation as in X-ray exposure standards.

For example, limits given in the Canadian Safety Code 6 (1991) [3] for occupational exposure to radio wave energy are 1 mW/cm² averaged over one hour period and 25 mW/cm² averaged over one minute period. They allow much higher pulse levels than the Swedish Standard [4] which even differentiates for the frequency range and has especially lower limits for the more damaging microwave range 5 mW/cm² 10-300 MHz averaged over 0.1h (=6 min.) 1 mW/cm² 0.3-300 GHz averaged over 0.1h (=6 min.) 25 mW/cm² 10 MHz-300 GHz averaged over 1 second.

Therefore the **cumulative exposure** is not being addressed in the ICNIRP guidelines and similar advice from WHO largely derived from the same scientists. The above limits only make sense in an environment where one is exposed to peaks or pulses of radiation especially in radar sites or to radiation during the operation of microwave equipment (ovens or heaters), which are switched on and off. Such values do not regulate how long one may be exposed to such electromagnetic fields, not even the maximum levels to which one is exposed.

With a pulse train with a 1:20 on/off ratio, one could be exposed to pulse energies proven to be highly dangerous - e.g. 500 mW/cm²- without violation of the Code. Also one could be exposed for a full second to 1.5 W/cm²over a one minute period. Early studies of cataractogenesis in relation to RF/MW exposure reported that 7% of this value caused eye damage (Paz, 1970s).

To be useful a Code has to give a peak limit **and** a dosage limit. Power from our electricity utilities is measured in Kilowatt-Hours, a unit used to measure accumulated power consumption over a time period . A unit for accumulated exposure to radiofrequency radiation should be established in the same manner, for example mWh/cm². If we use the exposure rates allowed by Safety Code 6 we get as an accumulated dose 1 mWh/cm²for one hour but 0.4 mWh/cm² for a minute allowed by the Swedish Code are for the 10-300 MHz range, and 5m Wh/cm² for the 300 MHz- 300 GHz range, the same as the Canadian Code. Only the maximal dose for one second which equivalates to 7 µWh/cm² may enforce lower peak values of exposure. But in both codes no values for accumulated doses are given and ICNIRP and other regulatory bodies have yet to come up with a dose that can be endured without damage, setting a radiation level that can be considered biologically safe for permanent exposure.

A different way of reviewing the RF/MW literature was provided by Dr Cindy Sage of Sage Associates Inc, a Montecito, California based consultancy. She showed that the ratio of studies reporting positive results far outweighed those reporting no biological effects (see Charts 1 and 2). Furthermore in confining the studies to those reporting chronic exposure effects it becomes evident that these too are far more likely to show effects than otherwise (Chart 3). Finally, the ratio of studies most likely to mimic cellphone or cellphone mast radiation levels also weighs heavily in favour of results reporting biological effects than those reporting none (Chart 4).

Chart 1: Radiofrequency Radiation Studies 1990 - 2003

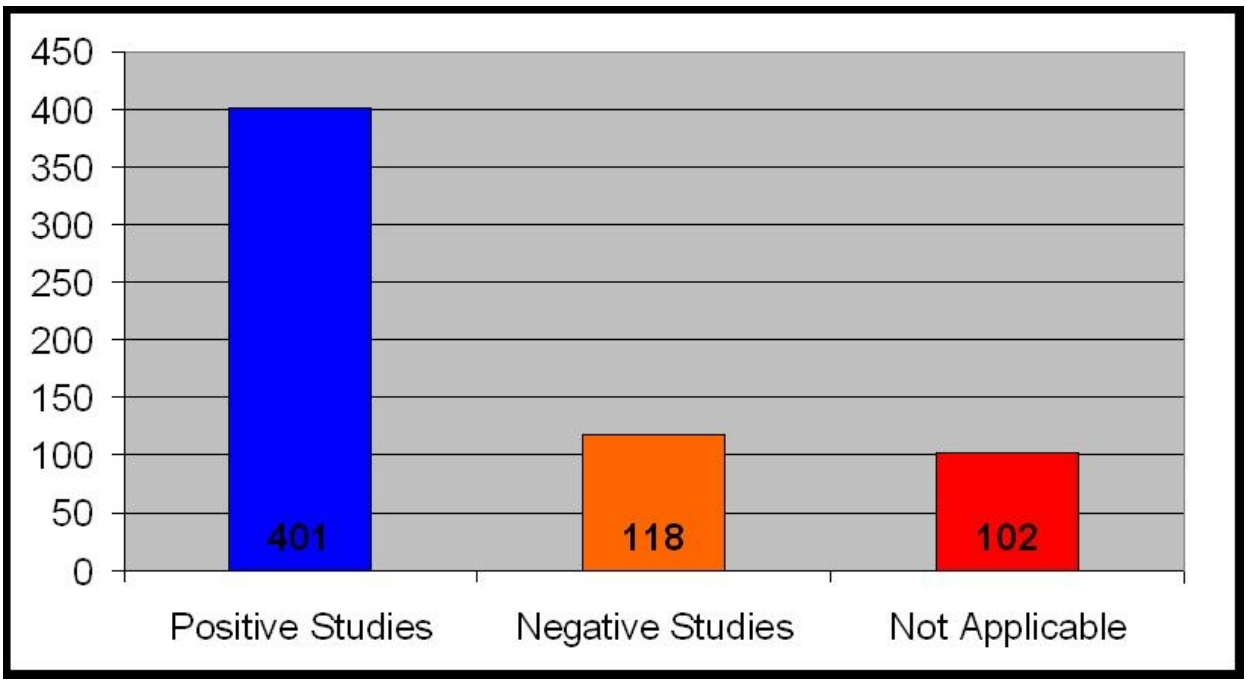
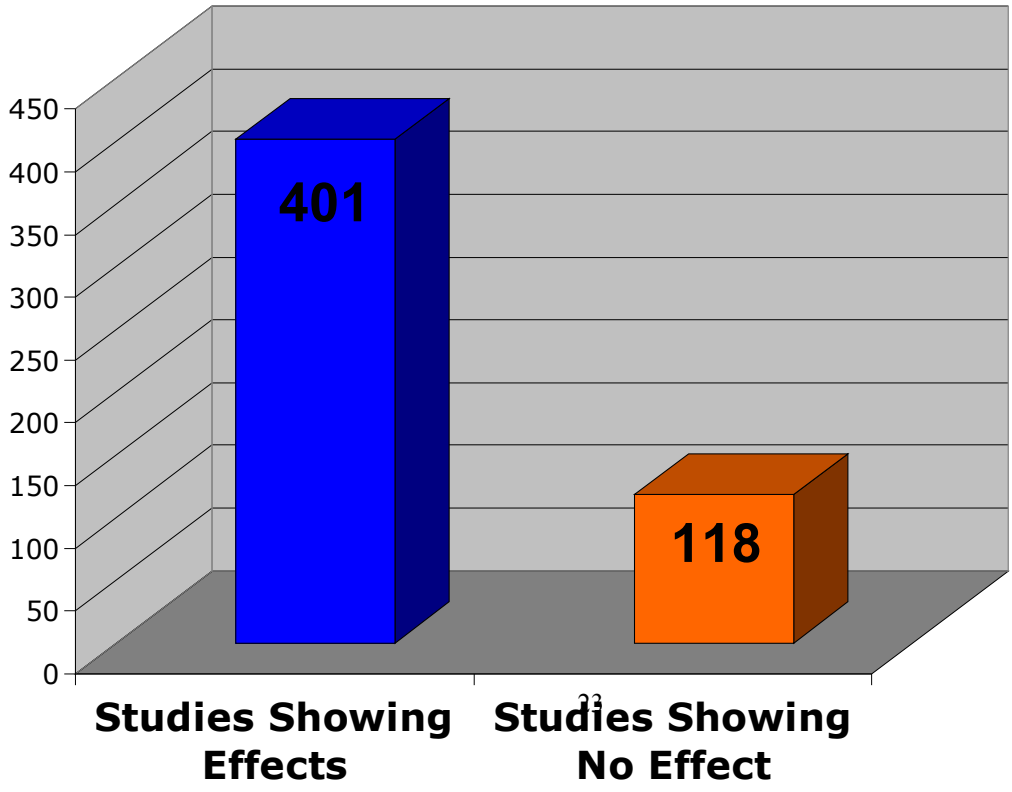


Chart 2: Radiofrequency Radiation Studies (1990-2004)



From this background one can now separate out the chronic studies. These show just how likely it could be that adverse health effects may only appear after some time, perhaps only after several years.

Chart 3: Radiofrequency Radiation Studies with Chronic Exposures (1990-2004)

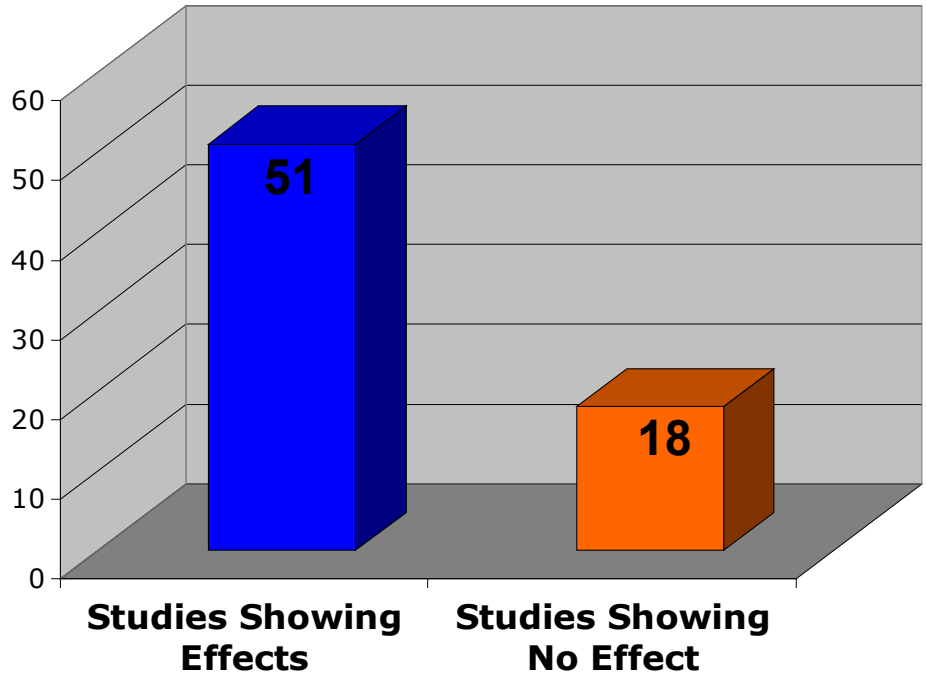
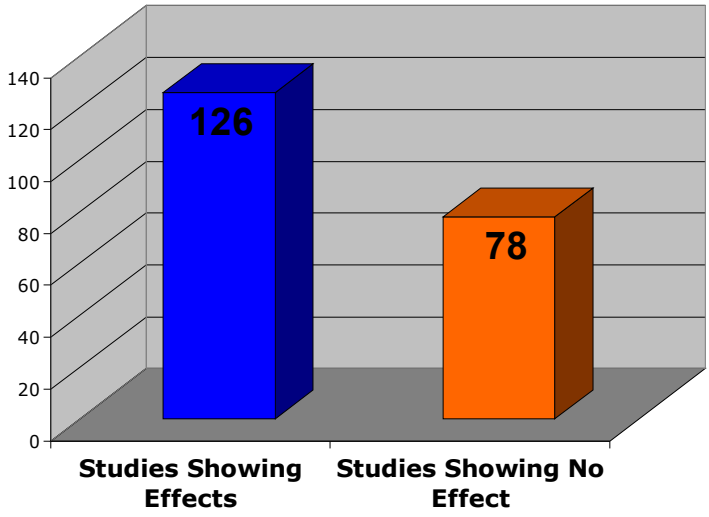


Chart 4: Studies Reporting Effects Similar to Cell Phone or Mast-Related Radiofrequency Radiation (1990-2004)



The issue of melatonin Inhibition by EMF

One fundamental issue emerging from the present literature is the effect of both power and radiofrequency on melatonin synthesis. This indoleamine is synthesised in several parts of the body as a defence against free radicals, particularly at night, when essential processes of cell repair and restoration via mitosis (cell division) take place. The adult human being repairs some half a billion cells in this way each night, and the energy essential for such repair is derived largely from oxidative phosphorylation. This in turn inevitably gives rise to free radicals, so evolution has arranged for the nocturnal synthesis of melatonin, a powerful free radical scavenger. Unfortunately it appears from studies at several laboratories that electric fields successfully inhibit melatonin synthesis. In consequence the potential damage to DNA from these free radicals is increased, thereby elevating the risk of cancer. Women with breast cancer have only one tenth the normal level of melatonin, and men with prostate cancer less than half compared with the normal population.

- Fourteen studies show that EMFs across the spectrum from ELF to RF/MW reduces melatonin in people.

- Wang (1989) who found that workers who were more highly exposed to RF/MW had a dose-response increase in serotonin, and hence indicates a reduction in melatonin. Abelin (1999) reported significant reductions from SW radio exposure, Burch et al. (1997) with a combination of 60 Hz fields and cell phone use and Arnetz et al. (1996) with VDTs.

- ELF exposure reduced melatonin in Wilson et al. (1990), Graham et al. (1994), Wood et al. (1998), Karasek et al. (1998), and Burch et al. (1997, 1998, 1999a), Juutilainen et al. (2000) and Graham et al. (2000); Pfluger et al. (1996)[16.7 Hz] and geomagnetic activity, Burch et al. (1999b).

Thus the possibility that exposure to RF/MW from cellphone masts causes a chronic reduction in melatonin cannot be ruled out.

In a relatively short letter such as this one cannot attempt a complete review of the literature, though competent and fairly recent attempts have been made by the late Prof Neil Cherry of Lincoln University New Zealand (2002) and by the late Dr Ross Adey, formerly of Loma Linda Veterans' Medical Center, (2005) and these are commended for further reading.

Scientific evidence reporting adverse health effects from weak chronic non-thermal exposure to RF/MW radiation continues to emerge, such as the recently published REFLEX report, a joint project of some dozen European laboratories who confirmed that weak RF/MW exposure can cause DNA damage, thereby confirming the earlier results of Lai and Singh. Such studies are to be found in the proceedings of Conferences (usually before they are later published in peer reviewed journals or in preprint format on the internet). Recent Conferences include the Bioelectromagnetics Societies annual meeting in Cancun, Mexico and the EHE06 Conference in Madeira Portugal (at both of which this author made presentations), with new Conferences shortly forthcoming in France (EBEA) and Crete.

Against this background it must be obvious that simply to rely upon the short term (acute) exposure guidelines offered by ICNIRP, which is evaporating in the present plethora of non-

thermal effects being reported, is inadequate for any planning authority. Heed must be paid to the overwhelming evidence that long term exposure to vanishingly weak RF/MW radiation will ultimately carry with it adverse sequelae to the health of vicinal exposees. In dealing prudently and applying the mandated precautionary approach advocated by the European Community's directives, planning authorities should seek to locate mast installations as distant as possible from human habitations.

Unfortunately the mechanism of protective free radical scavenging action by melatonin is still not well understood, but it could well operate as a reactive chain reaction. Plant-derived melatonin has also recently been shown to inhibit the damaging effects of intra-mitochondrial peroxynitrite at less than 200μg/ml for example (Basu and Hasra, 2006), and natural compounds and polyherbal formulations to act radioprotectively even against ionising radiation (Maurya, Devaagayam et al., 2006). Other recent studies report synthesis of this free radical as well as nitrites and nitrates from exposure of aerated biological fluids to weak electric fields.

Melatonin is also known to upregulate the expression of other anti-oxidants (Mayo, Sainz et al., 2002: and see Hardeland and Pandi-Perumal (2005) for recent review). This characteristic is also therefore a candidate for a causal mechanism of interaction at ultra low concentrations.

The homeostatic role of nitric oxide in controlling oxidative phosphorylation is also assisted by melatonin, suggesting its action is at a submolecular level. Finally many studies confirm that the normal peak level of melatonin in human serum is in the 20-120 picogram/ml range, making it the smallest of all hormones in terms of body volume. Against this background the potential ability of melatonin to act effectively at very minute doses cannot be ruled out.

CONCLUSIONS

This is the first clinical trial to investigate the somniferous action of plant-derived melatonin at picogram/ml administration levels on humans. We conclude that these results, significant at the $p=0.01$ level, show a sufficient trend to support the view that melatonin can exert a somniferous effect at levels far below current pharmacological doses, and that Asphalia is a suitable candidate as a melatonin delivery agent at these low concentrations. Further *in vitro* work is necessary to understand better the mode of action from among several plausible candidates, and to investigate its effect *in vivo* on younger and more elderly groups than those considered here.

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Competing interests:

RWC is a director of Medcross Ltd., the ultimate holding company of Asphalia.

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APPENDIX A: Questionnaires

Questionnaire 1

To be completed BEFORE starting Asphalia

All information given on this questionnaire will be treated in the strictest of confidence. Please supply us with as much information as you feel comfortable to give.

Asphalia Kit Number:……..

Code:……

Sex : Male / Female

Age:……years…..months

Town and County of residence:…….

Length of time at current address:…....years……..months

Proximity of home to Masts/Pylons/Substations:…….metres

Length of time Masts/Pylons/Substations have been present:……years…..months

Occupation:
……
……

Length of time in current employment:……years……months

Proximity of workplace to Masts/Pylons/Substations:……metres

Length of time Masts/Pylons/Substations have been present:……years…..months

What equipment do you use at work, please included duration of exposure (hours and minutes):
……
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……
……

Do you use a mobile phone?: Yes / No

How often do you use a mobile phone per day?:
Number of calls daily…….
Average duration of calls……minutes

What make/Model is the mobile phone?:
…….

Do people in your household use a mobile?: Yes / No

How often do they use their mobile in your presence?:
Number of calls daily…….
Average duration of calls……minutes

Do you smoke? Yes/No
If yes how much daily?:……..
If yes will you be continuing or giving up for this trial?: Yes/No

Briefly describe your medical history, include dates and duration of illness where possible:
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Briefly describe symptoms you believe are linked to exposure to masts/powerlines etc:
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Please use space overleaf to add any other points you feel may be of use:

Please tick box if you have added information overleaf	

Questionnaire 2

Please complete two weeks after the start of you course of Asphalia

All information given on this questionnaire will be treated in the strictest of confidence, please supply us with as much information as possible that you feel comfortable to give.

Asphalia Kit Number:……..

Code:……

Sex : Male / Female

Age:……years…..months

Town and County of residence:…….

Using the list below please describe your symptoms since taking Asphalia. Tick the box which applies to you.

	Extreme worsening	Moderate worsening	Slight worsening	No change	Small improvement	Moderate improvement	Extreme improvement	Not applicable
Headache								
Asthma/breathing								
Sleep disturbance								
Memory loss								
Numbness								
Depression								
Nose bleed								
Nausea								
Emotional lability								
Lack of concentration								
Other (please state)								

Have you taken Asphalia as directed, i.e. have you missed any doses, taken them later or earlier than usual etc:

……
……
……

Please use space overleaf to add any other points you feel may be of use:

Please tick box if you have added information overleaf	

Questionnaire 3

Complete at the end of your course of Asphalia

All information given on this questionnaire will be treated in the strictest of confidence, please supply us with as much information as possible that you feel comfortable to give.

Asphalia Kit Number:……..

Code:……

Sex : Male / Female

Age:……years…..months

Town and County of residence:…….

Using the list below please describe your symptoms since taking Asphalia. Tick the box which applies to you.

	Extreme worsening	Moderate worsening	Slight worsening	No change	Small improvement	Moderate improvement	Extreme improvement	Not applicable
Headache								
Asthma/ breathing								
Sleep disturbance								
Memory loss								
Numbness								
Depression								
Nose bleed								
Nausea								
Emotional lability								
Lack of concentration								
Other (please state)								

Have you taken Asphalia as directed, i.e. have you missed any doses, taken them later or earlier than usual etc:

……
……
……
……
……

Overall, over the course of Asphalia would you say that:
(please circle the relevant letter)

a) I have felt no difference in my health/symptoms
b) I have felt a slight improvement in my health/symptoms
c) I have felt an improvement in my health/symptoms
d) I have felt a considerable improvement in my health/symptoms
e) I have felt a worsening of my health/symptoms
f) I have felt a slight worsening of my health/symptoms
g) I have felt a considerable worsening of my health/symptoms

Apart from the taking of Asphalia and the recommended diet, have you made any other major or minor lifestyle changes?:

……
……
……
……
……

Please use space below and overleaf to add any other points you feel may be of use:

Please tick box if you have added information overleaf	

# # #