ΦΑΚΕΛΟΣ ΕΡΕΥΝΗΤΙΚΩΝ ΔΡΑΣΤΗΡΙΟΤΗΤΩΝ ΤΗΣ ΜΕΛΕΤΗΣ ΤΗΣ ΕΠΙΔΡΑΣΗΣ ΣΤΟΧΕΥΜΕΝΩΝ ΗΛΕΚΤΡΟΜΑΓΝΗΤΙΚΩΝ ΠΕΔΙΩΝ ΣΕ ΕΜΒΙΑ

6

Δημοσιεύσεις και ανακοινώσεις αποτελεσμάτων της επίδρασης στοχευμένων Ηλεκτρομαγνητικών πεδίων σε διεθνή περιοδικά και διεθνή επιστημονικά συνέδρια.

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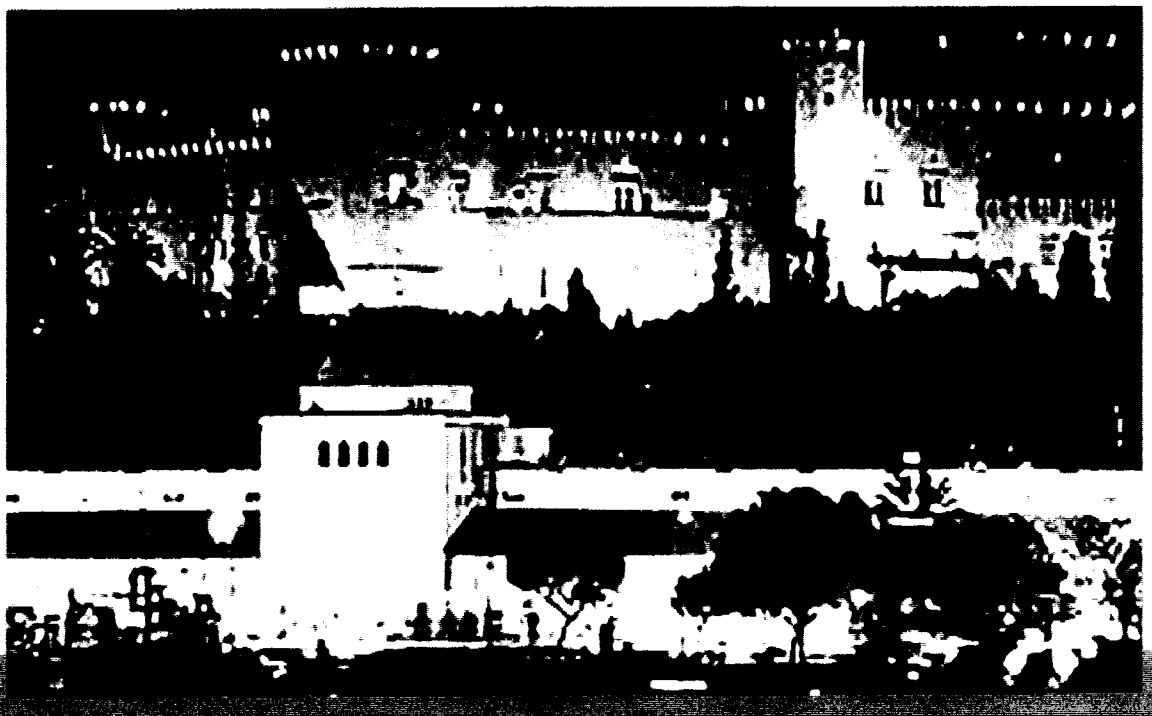
Abstracts



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P-70 Tumor Biology

Electromagnetic (EMG) signals of nerve growth factor (NGF) may induce differentiation of rat pheochromatocytoma cells PC 12

Karkabounas[1] Spiros C, Scarpelis[1] G, Havelas[1] K, Metsios [1] A, Hadjiavazis[1] G, Giotis[2] C and Evangelou[1] AM
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Aims: In this study we investigated whether EMG signals may induce differentiation of PC12 cells.
Methods: The NGF EMG signals were recorded by a device emitting EMG waves and recording the resonance to the waves emitted by the substance tested. Emission of NGF signals was performed by *Multi Channel Dynamic Exciter 100 VI* utilizing an EMF generator of RW, at various intensities. The PC12 cells were cultured for 10 days and then in a Faraday cage: 1.Experimental sample (ExS) cells were exposed to static EMF signals of NGF, for 12 hours a day for 3 consecutive days, 2. Control sample (CS) cells were exposed with the device switched off, for the same period of time. Comparison samples (COS) of cells were cultured in presence of human NGF at various concentrations (1to20 μg/ml).
Results: A band of frequencies between 10 kHz to 200 kHz, was recorded for NGF. **CS cells** showed a slight increase in proliferation rate with no morphological changes at the end of the experiment. **COS cells** showed a dose dependent increase of its proliferation rate and differentiation in nervous cells.**ExS** cells showed no significant increase of proliferation rate after the exposure to the signals of NGF. After the end of 3rd exposure a high percentage (>50 %) of PC12 cells presented morphological features of nervous cells and a formation of neuronal networks. Repeated cultures of the EMF exposed cells revealed that they conserve their differentiation features for long, while, if no NGF is added in the culture media of the comparison samples, cells abolish their differentiation within 3 days.
Conclusions: NGF as other substances[1] emit signals of EMG nature that, if transmitted to target cells (PC12), may cause their differentiation to nervous cells, similar and more permanent to that the substance itself can do.

P-71 Tumor Biology

Somatic mutations and activation-induced cytidine deaminase (AID) expression in a rheumatoid factor producing lymphoblastoid cell line

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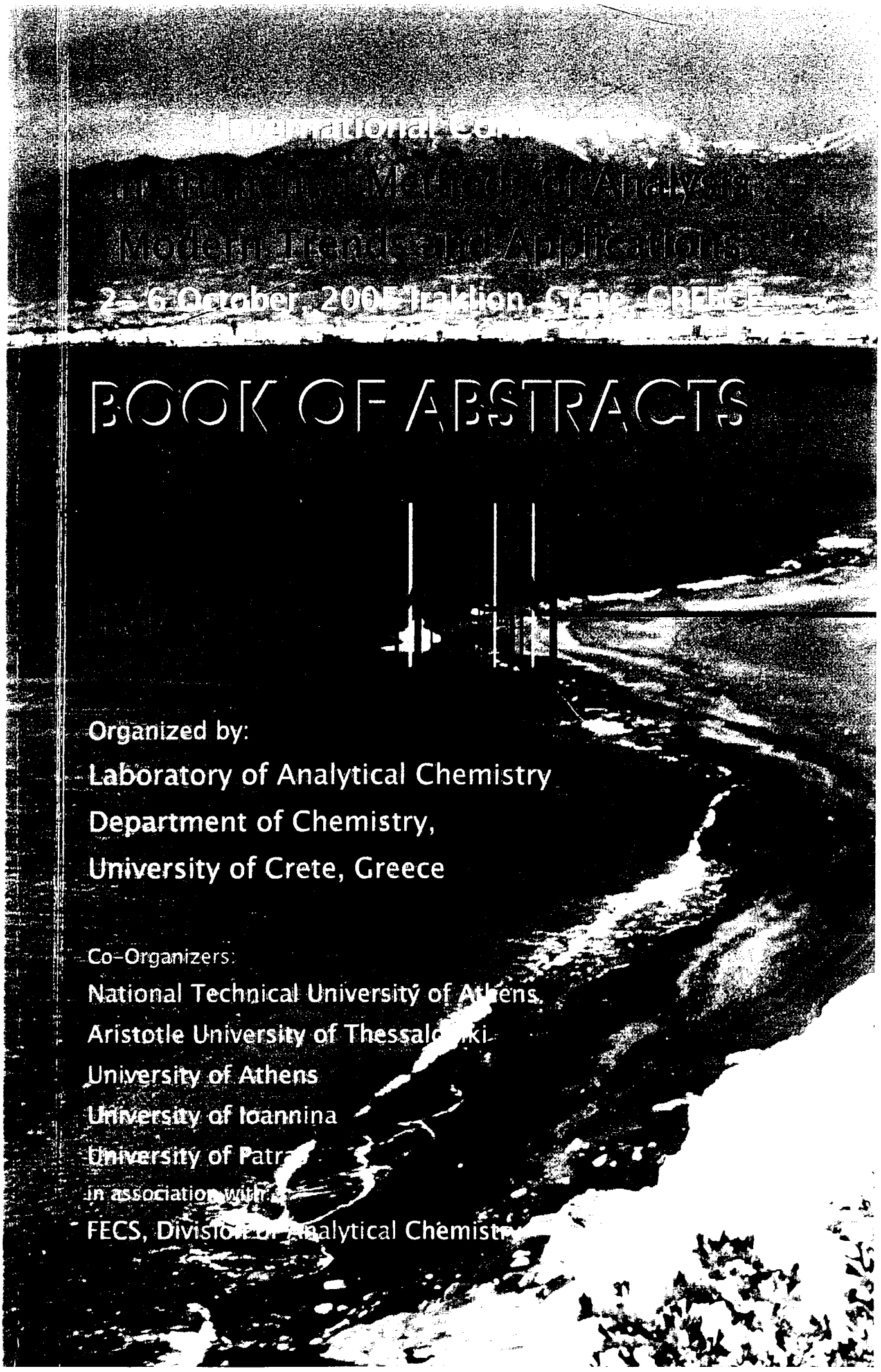
The Hebrew Univ-Hadassah Medical School, Jerusalem, Israel

Aims: The question whether Epstein-Bar virus (EBV) transformed lymphoblastoid cell lines (LCLs) exhibit somatic mutations in their Ig variable region genes (IgV) during in vitro growth was studied.
Methods: The sequences of the rearranged V_H of an adult-LCL which secretes a monoclonal IgM rheumatoid factor (RF-line) and of the V genes of cord blood LCLs were determined.
Results: EBV infection of adult and cord blood lymphocytes induces a rapid induction of AID, a mutator responsible for somatic hypermutation (SHM) in the IgV. SHM were not found in the rearranged V of cord blood LCLs. By contrast, the rearranged V_H gene of the RF-line, exhibited a low level of somatic mutations in culture. The mutations were preferentially targeted to the WRCH/DGYW hot spot motifs and biased for GC nucleotides, indicating that they were due to AID mediated SHM. Two point mutations in the CDR1&2 of the V_H of "non-antigen binding" RF clones, correlated with loss of antigen binding activity.
Conclusions: Induction AID expression and SHM in the rearranged V_H of adult-LCL, may explain the occasional loss of antigen binding activity occurring in freshly established antibody secreting LCLs. In addition, our results support the possibility that AID may act as an oncogene, since the tumorigenic outcome of EBV infection in B-cells, may be partly mediated by the induction of the mutatory activity of AID.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P-I-26

EFFECTS OF LOW INTENSITY STATIC ELECTROMAGNETIC FIELDS (SEMF) ON SARCOMA CELL LINES IN VITRO.

S. Karkabounas[a], P. Veltsistas[b], D. Stergiou[b], J. Binolis[a], G. Hadjiavazis[a], A. Zouridakis[a], A. Metsios[a], N. Papadopoulos[a], K. Havelas[a] and A. Evangelou[a]*

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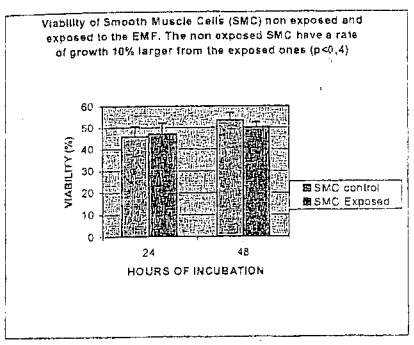
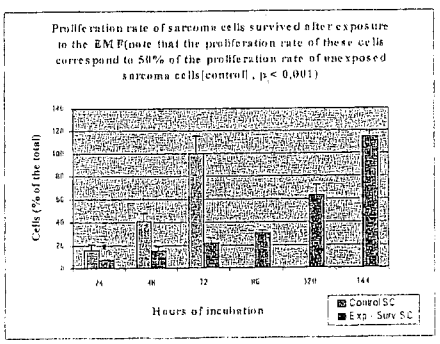
In this study we have investigated the effects of Static Electro-Magnetic Fields (SEMF) on sarcoma cells, isolated from tumors of Wistar rats. The tumors were developed by 3,4-benzopyrene (B[α]P) injection in the rats. The cancer cells were exposed to SEMF for 45 minutes, applying frequencies between 10 kHz to 1 MHz, of the radiowave spectrum. During a 24-hours period after cancer cell exposure to SEMF, no inhibition of cell proliferation appeared. In contrast, at the end of 48 hours incubation time, the cancer cell proliferation was dramatically decreased at a level of 98%.





In addition the survived sarcoma cells, which are 2% of the total cell population, after its exposure to SEMF, showed a significant decrease of proliferation, under the same culture conditions. These cells were then exposed once again to SEMF for 45 minutes (totally 4 sessions of exposure) and tested using a Flow Cytometer. It was found that a great percentage of these cells (45%), doubly exposed to SEMF, was apoptotic and only a small percentage of them was found under mitosis (2 %). Additionally, the cells were counted and tested, by using an aggregometer for their ability to aggregate the platelets (an indicator of their metastatic potential) and they didn't show any difference, compared to the sarcoma cells not exposed to SEMF (control cells).

P-I-27

DIFFERENTIATION EXPERIMENTAL EFFECTS OF LOW INTENSITY SEMF, ON PHEOCHROMOCYTOMA CELLS, TYPE PC-12.

S. Karkabounas, [b]P. Veltsistas[a], D. Stergiou[b], G. Scarpelis[a], G. Hadjiavazis[a], A. Metsios[a], N. Papadopoulos[a], K. Havelas[a] and A. Evangelou[a]*

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Herein we investigate the effects of the Static Electro-Magnetic Fields (SEMF) on the pheochromocytoma cell line PC-12, isolated from a rat's pheochromocytoma. The PC-12 cells colony was incubated for 10 days at 37 °C (O2 95%, CO2 5%) and then exposed to SEMF, 12 hours every day, in 3 consecutive sessions, by using Radio Wave Frequencies, between 10-200 kHz of radiowave spectrum. Radio frequency measurements and SEMF exposure of cells were performed by MULTI CHANNEL DYNAMIC EXITER 100-V1, a device certified by the International Committee of Atomic Energy-EKEFE-DEMOKRITUS, for its safe use in humans and animals. It consists of two main parts: a) a diagnostic part with EPR-spectrometer characteristics and b) a SEMF generator of varying intensities (1.1- 1.11 ± 0.01 V/m for electric field and 0.0027-0.0029 ± 0.00005 A/m for magnetic field), along with radio frequencies (1kHz to 1MHz), monitored by a sophisticated software. To use this software, first it is necessary to record the biological target system's frequencies and then, by using a specific algorithm, to calculate the appropriate SEMF frequencies, that are needed for the exposure of living target systems or cells.

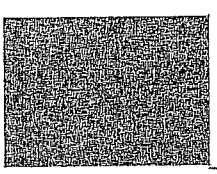

Fig. 1

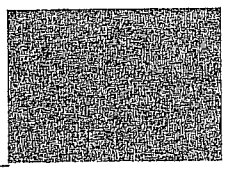

Fig. 2

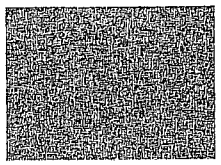

Fig. 3

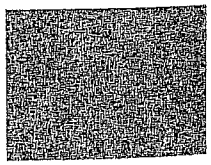

Fig. 4

A slight inhibition of cell proliferation rate was observed after exposition to SEMF. After first exposition a small number of PC-12 cells, presented morphological characteristics of nervous cell differentiations, (Fig. 1, Fig. 2). At the end of the third exposure session, a high percentage (>50%) of the PC-12 cells presented conspicuous morphological characteristics, of nervous cells and a formation of well-described neuronal networks (Fig. 3). It's not yet clear if this

P-I-36

DETERMINATION OF ANTICANCER ACTIVITY OF RESVERATROL ON CANCER CELLS, BASED ON THE CYTOMETRIC MONITORING OF THE NK LYMPHOCYTES STIMULATION

T. Daskalou [a], S. Karkabounas[a], I. Toliopoulos[a], D. Bougiouklis[a], A. Evangelou[a], A.Metsios[a], P. Veltsistas[b] and S. Gerou[c].

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Introduction: Natural Killer Cells (NK Cells - NKCs) are a subpopulation of lymphocytes that play an important role in immunotherapy. Resveratrol (3,4,5-trihydroxy-stilbene-3-β-D-glucoside) is an ingredient of many plants, with antioxidant properties.

Purpose: The investigation of possible anticancer actions of resveratrol, by stimulation of NK cells.

Material and Methods: 18 healthy volunteers participated in the study. The methodology of quantification of cytotoxicity of NKCs was used in the in vitro study, which included four stages: a) isolation of NKCs from blood and their quantification, b) quantification of cancer cells (leiomyosarcoma - Wistar rats), which used as cancer target cells (CTCs), c) incubation of NKCs with CTCs in CO_2 chamber in the ratios 12.5:1, 25:1, and 50:1 and d) determination of cytotoxicity by flow cytometer Epics XL-MCL of Beckman-Coulter Co. The same trials were repeated after the addition of resveratrol during stage c.

Results: The cytotoxicity of NKCs against CTCs indicated an increase at 320%, 440%, 67% average rate in the ratios 12.5:1, 25:1 and 50:1 respectively.

Conclusions: Resveratrol seems to be an important anticancer substance and therefore further clinical studies should be performed, for more convenient prevention and therapy of cancer.

P-I-37

AGGREGOMETRIC DETERMINATION OF ANTITUMOR AND ANTIPLATELET POTENCY OF CARVACROL, FOR THE PREVENTION OF CANCER AND THROMBOEMBOLIC DISEASES

T. Daskalou[a], S. Karkabounas[a], I. Toliopoulos[a], D. Bougiouklis[a], A. Evangelou[a], A.Metsios[a], P. Veltsistas[b] and S. Gerou[c].

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Introduction: Carvacrol is a basic ingredient of ethereal oil of *Origanum Vulgaris sbsp Hirtum.*

Purpose: The investigation of possible anticancer and antiplatelet actions of carvacrol (5-isopropyl-2-methylphenol).

Materials and Methods: i) anticancer action: in 28 healthy volunteers the cytotoxicity of NK cells was checked with the methods of cytotoxicity assay and flow cytometry with the use of carvacrol (10^{-3} M), ii) platelet aggregation (PA): in the isolated platelet rich plasma (PRP) of 28 healthy volunteers, trials of PA were performed with the stimulators ADP, PAF and arachidonic acid (ArA) in the presence and absence of carvacrol in concentrations 10^{-4} to 6.5 x 10^{-3} M, in aggregometer Ca-500 of Chronolog Co.

Results: i) the increase of cytotoxicity observed was 110% in average in the ratio 25:1, while the cytotoxicity in the ratios 12.5:1 and 50:1 remained stable, ii) the PA caused by ADP, PAF, and ArA was completely inhibited when carvacrol was added in concentrations such as 6.5 x 10^{-3}, 4.5 x 10^{-3} and 6.5 x 10^{-4} M respectively.

Conclusions: Carvacrol, a substance with antioxidant properties develops anticancer and antiplatelet actions. Therefore, with the addition of clinical trials, it could be useful in the therapy and prevention of cancer and thromboembolic diseases.

P-I-38

AGGREGOMETRIC - CYTOMETRIC MONITORING OF THE ACTION OF VARIOUS ANTIOXIDANTS ON PLATELET ACTIVITY, FOR THE CLINICAL PREVENTION OF THROMBOEMBOLIC DIDEASES

T. Daskalou[a], S. Karkabounas[a], I. Toliopoulos[a], D. Bougiouklis[a], A. Evangelou[a], A.Metsios[a], P. Veltsistas[b] and S. Gerou[c].

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Introduction: Membranic platelet receptor GpIIb-IIIa contributes to platelet aggregation (PA) by binding fibrinogen. Administration of antioxidants (AO) to platelets inhibits PA.

Purpose: To investigate the possible inhibition of: a) PA and b) the operation of GpIIb-IIIa ex vivo, through administration of AO.

Materials and Methods: 28 healthy volunteers participated in the study as blood donors. The following AO were administered in their rich platelet plasma (PRP) in concentration 3 x 10^{-3} M per substance: 2,3-diphosphoglyceric acid (2,3-DPG), carvacrole, gloutathione (GSH), azoulene, bismuthiole, 2-methyl-2-nitroso-propane (MNP) and N-tert-butyl-α-phenylnitrone (PBN). The GpIIb-IIIa receptors were measured by ADIAflo Platelet Occupancy kit, American Diagnostica Inc. and the flow cytometer Epics XL-MCL-Beckman Coulter. PA tests to the PRP were implemented with epinephrine (EPN), thrombine (THR), arachidonic acid (AA), PAF and ADP as activators. Same tests took place after administration of the under study AO in the PRP at 3 x 10^{-3} M concentration per substance. PA was calculated in a PICA Chronolog Co aggregometer.

Results: After the administration of the AO 2,3- DPG, carvacrole, GSH, azoulene, bismuthiole, MNP and PBN: a) the operation of the receptor GpIIb-IIIa decreased by 92, 99.4, 93, 91.5, 90, 95, and 89 % respectively and b) inhibition of PA was provoked at 87, 94, 88, 84, 83, 91 and 82% respectively.

Conclusions: Antioxidants contained in many plants and fruits and known to be free oxygen radicals scavengers, are possible to act at the level of platelet receptors GpIIb-IIIa inhibiting their function and in this way averting the configuration of platelet clotting. Based on these given facts, they could be used complimentary to the prevention of thromboembolic diseases.

P-I-44

FLOW CYTOMETRIC MONITORING OF THE APIGENIN ACTIVITY ON THE INDUCTION OF NK CELLS' FUNCTIONALITY, FOR THE CLINICAL PREVENTION OF CANCER

T. Daskalou[a], S. Karkabounas[a], I. Toliopoulos[a], D. Bougiouklis [a], A. Evangelou [a], P. Veltsistas[b] and S. Gerou[c].

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Introduction: Natural killer cells (NK Cells – NKCs) are a subpopulation of lymphocytes that play an important role in immunotherapy.

Purpose: The investigation of possible induction of functionality of NK cells by the use of apigenin (4',5,7-trihydroxyflavone).

Materials and Methods: 18 healthy volunteers participated in the study. The methodology of quantification of cytotoxicity of NKCs was used in the in vitro study, which included four stages: a) isolation of NKCs from blood and their quantification, b) quantification of cancer cells (leiomyosarcoma - Wistar rats), which were used as cancer target cells (CTCs), c) incubation of NKCs with CTCs in CO_2 chamber in the ratios 12.5:1, 25:1, and 50:1 and d) determination of cytotoxicity by flow cytometer Epics XL-MCL of Beckman-Coulter Co. The same trials were repeated after the addition of apigenin during stage c.

Results: The cytotoxicity of NKCs against CTCs indicated an increase of 320%, 480%, average rate in the ratios 25:1 and 50:1, while no increase in cytotoxicity observed in the ratio 12.5:1.

Conclusions: Apigenin seems to have important anticancer properties against cancer cells and its use in clinical trials should be seriously considered in the future.

P-I-46

CYTOMETRIC MONITORING OF PHYTOESTROGENES EFFECTS ON PLATELET ACTIVITY

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[c]"ANALYSI", Microbiological and Biomedical Research Lab., 54623, Thessaloniki, GREECE.

Introduction: Membranic platelet receptor GpIIb-IIIa participates in platelet aggregation (PA) as fibrinogen's receptor. Administration of phytoestrogens (PO) to platelets inhibits PA.

Purpose: To investigate the possible inhibition of a) PA and b) the operation of GpIIb-IIIa ex vivo, through administration of PO.

Materials and Methods: 28 healthy volunteers participated in the study as blood donors. The PO 1) apigenine (4',5,7-trihydroxyflavone), 2) β-naphthol (β-hydroxynaphthalene), 3) quercetin (3,3',4',5,6,7-hexahydroxyflavone), 4) resveratrol (3,4',5-trihydroxy-stilbene-3-β-D-glucoside), 5) thymol (3-hydroxy-p-cymene), 6) genistein (4,5,7-trihydroxy-isoflavone) and 7) origan oil were administered in their rich platelet plasma (PRP) at a concentration of 3×10^{-3} M per substance. The GpIIb-IIIa receptors, were measured by ADIAflo Platelet Occupancy kit, American Diagnostica Inc. and the flow cytometer Epics XL-MCL-Beckman Coulter. PA trials to the PRP were implemented with epinephrine (EPN), thrombine (THR), arachidonic acid (AA), PAF and ADP as stimulants. Same trials took place after administration of the under study PO in the PRP at a 3×10^{-3} M concentration per substance. PA was calculated in a PICA Chronolog Co aggregometer.

Results: After the administration of the PO apigenine, β-naphthole, quercetin, resveratrol, thymol, genistein and origan oil: a) the operation of the receptor GpIIb-IIIa decreased by 91, 98.1, 93, 91, 92, 89 and 99.5 % respectively, b) each substance studied caused a 100% inhibition of PA.

Conclusions: Phytoestrogenes contained in many plants and fruits and known to be free oxygen radicals scavengers, are possible to act at the level of platelet receptors GpIIb-IIIa inhibiting their function and in this way averting the configuration of platelet clotting. Based on these facts, they could be used complimentary, to prevent thromboembolic diseases.

P-I-47

KINETIC AND POTENTIOMETRIC ASSAY OF FORMALDEHYDE IN REAL SAMPLES, MONITORED BY COPPER SOLID ION SELECTIVE ELECTRODE, AFTER ITS REACTION WITH $[CU\{(CH_2NH_2)_2\}_2]\cdot SO_4$.

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Many publications have been reported so far, regarding electroanalytical methods for HCHO determination[1]. HCHO reacts quickly, quantitatively, irreversibly and stoicheiometricaly (2/1), with aquatic solutions of the stable complex Bis-(Ethylenediamino)-Cu(II)-Sulfate, thus demasking Cu(II) cations and producing the soluble ethylenediimine, according to the reaction sheme[2]:

$$[Cu\{(CH_2NH_2)_2\}_2]\cdot SO_4 + 4HCHO \rightarrow Cu^{2+}_{(sol)} + SO_4^{2-}{}_{(sol)} + (CH_2)_8N_{4(sol)} + 4H_2O$$

The Cu(II)-solid membrane ISE, type OP-CU-0711P RADELKIS[3] is monitoring Cu(II) cations, being released and kinetic curves are taken. Their initial slopes, as well as their limiting potentials linearly correlate with HCHO concentrations. Calibration graphs are taken in concentration range of 50-250 ppm HCHO, at an optimum pH 7. The main advantages of the proposed method are the simplicity, the low cost and the speed of the measurements. It is recommended for HCHO assay, in relatively concentrated samples 0.02-0.04 M, as well as in diluted solutions 0.001 M. It can, also, be successfully applied in colored and turbid samples or emulsions, where other methods fail.

P-I-54

KINETIC-CATALYTIC MICRO-ASSAY OF FE(III), ON HUMAN SERUM AND PHARMACEUTICAL SAMPLES, BASED ON THE PERBROMATE - DIPHENYLAMINE REACTION, IN A MIXED ACID MEDIUM.

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Perbromic acid ($HBrO_4$) is a strong monobasic acid and very strong oxidant at 100 °C, but almost inactive at room temperature. Thermodynamic and kinetic limitations make synthesis of perbromates[1] almost inevitable, under usual conditions. Due to their lack, a few papers have been reported so far among the scientific community, concerning the development of spectrophotometric[2], kinetic[3] and potentiometric methods[4]. The water insoluble Diphenylamine (DPA), becomes soluble in concentrated mineral and strong organic acids. Here in we describe the development of a novel kinetic spectrophotometric determination of Fe(III), based on its catalytic effect on the reaction of perbromates with diphenylamine in a mixed acid medium.

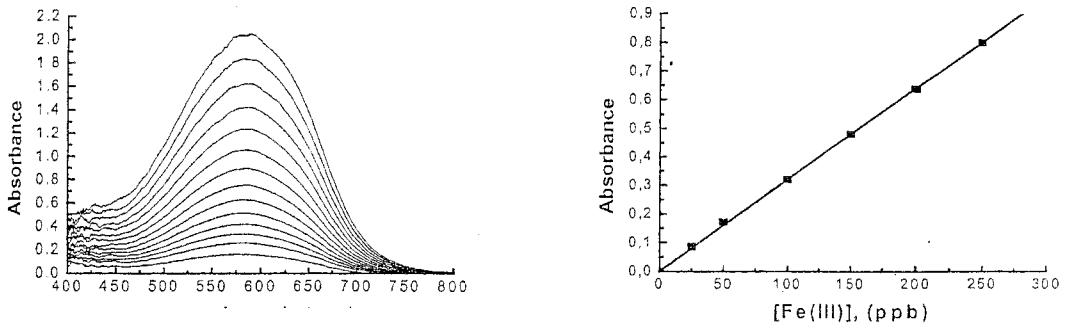

FIGURE: LEFT: The visible spectrum progress of the blue product, every 30 sec. DPA: 50 μl (0.5% stock solution), $KBrO_4$: 10 μl (0.1 M stock solution), Fe(III): 250 ppb, Final volume: 2 ml mixed acid. RIGHT: Calibration curve for the determination of Fe(III), according to the proposed method, in the concentration range 25-250 ppb.

P-I-68

SYNTHESIS, CHARACTERIZATION AND X-RAY STRUCTURE ANALYSIS OF A NOVEL POLYOXO-AZIDE, "BALLERENE" TYPE COMPLEX, WITH MIXED VALENCE STATES OF VANADIUM (IV)-(V).

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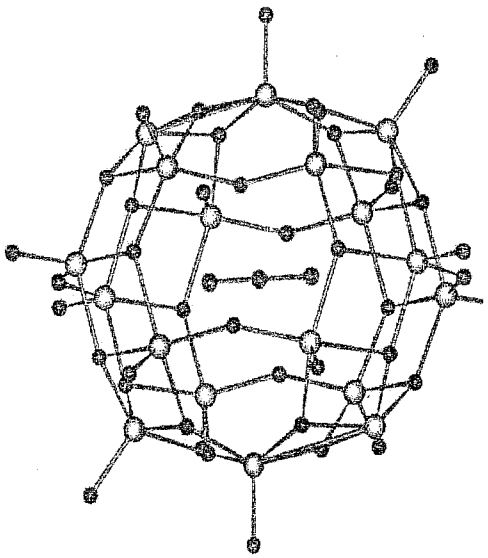
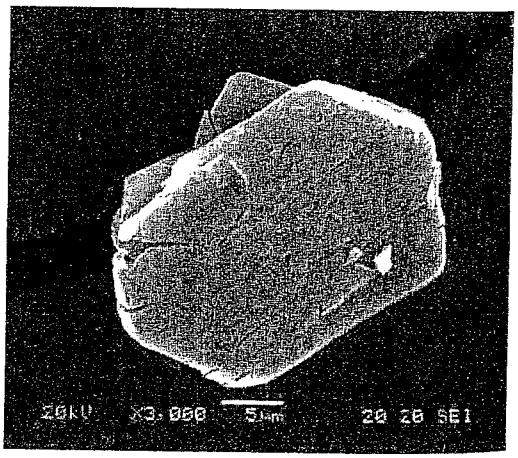
Vanadium is an essential trace element, with relevant biological and therapeutical properties, therefore it posses a special status among all biometals. A variety of vanadium compounds have been synthesized so far in an effort to offer better tolerance, more potent activity, increased selectivity and less toxicity in cancer treatment[1]. Thus many research groups have focused in the synthesis of novel vanadium compounds[2], which have been extensively reviewed[3]. In our effort





to prepare new electroactive amperometric mediators, we study the electrochemical behaviour of the binary system NH_4VO_3-NaN_3, before and after thermal treatment[3]. Finally we managed to isolate for a first time the ballerene-type cluster, with catenes hyphenated by V(IV)-V(V) strong interactions, along with a $[N_3^{\cdot}]$ radical located at the center of the cluster, as it can be seen in the picture above. The cluster with the molecular formula $Na_{10}[V_{18}O_{44}N_3]\cdot 33H_2O$, is centrosymmetric and crystallizes in the triclinic P-1 space group, with cell parameters: a=12.019 Å, b=13.114 Å, c=13.425 Å, α=114.77°, β=92.86°, γ=113.93°, V=1693.44 $Å^3$. There are two

EFFECTS OF LOW INTENSITY RADIOFREQUENCY STATIC ELECTRO-MAGNETIC FIELDS (EMFs), ON SARCOMA CELL LINES.

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Abstract

In this study we investigated the effects of low intensity static electromagnetic field (EMF) causing no thermal effects, on sarcoma cells, isolated from tumors of Wistar rats. The tumors were developed via 3, 4-benzopyrene injection in the rats. The cancer cells were exposed to EMF using frequencies between 10 kHz to 120 kHz of the radiowave spectrum for 45 minutes. During a 24-hours period after cancer cell exposure to EMF, no inhibition of cell proliferation appeared. In contrast, at the end of 48 hours incubation time, the cancer cell proliferation was dramatically decreased in ratio > 95%. Also, the survived sarcoma cells after the exposure to EMF (2% of the total cell population exposed to EMF) showed a significant decrease to proliferate under the same culture conditions. These cells were then exposed once again to EMF for 45 minutes (totally 4 sessions of exposure) and tested using a flow cytometer. It was found that a great percentage (45%) of these cells, double exposed to EMF, was apoptotic and only a small percentage of them was found under mitosis (2 %). Additionally, the cells were counted and tested using an aggregometer for their ability to aggregate the platelets (an indicator of their metastatic potential) and they didn't show any difference in comparison to the sarcoma cells not exposed to EMF (control cells).

Key Words: Sarcoma cells, Static electromagnetic fields, radiofrequency waves , resonance, platelet aggregation, metastasis.

Abbreviations : Electromagnetic fields : EMF , Smooth Muscle cells : SMC, Malignant sarcoma cells : MC, SC, Platelets Reach Plasma : PRP , 3,4-benzopyrene : B[a]P.: DMEM , Fetal Bovine serum : FBS , Radio – frequencies : RF, Electron Paramagnetic Resonance : EPR , Electron Spin Resonance : ESR.

Introduction

There is a lot of data dealing with the effects of electromagnetic fields (EMFs) on cells, experimental animals and humans, some of them referred to application of electromagnetic resonance principles(1, 2). Dealing with malignancy , the following main concepts have been expressed, so far, depending on the intensity, frequency and duration of application of the electromagnetic waves : The EMFs may act as co-carcinogens in combination with the initiating carcinogen , especially in experimental animals and the EMFs can exert anticarcinogenic effects, inhibiting the proliferation of malignant cells in vitro as well as decreasing the size of the experimental tumors in vivo (3,4,5,6). The studies on EMFs pro-carcinogenic effects in experimental animals are however, not numerous and it seems that the described methods have a lot of uncertainty (3,4). In comparison, the studies on EMF anticancer effects are abundant and their methodology is well documented (7). It has been also shown, that the cytostatic effects of the EMFs on cancer cells are not related to their thermal effects but are exerted via temperature-independent actions(8,9,10).

In the present study the effects exerted by low intensity radiofrequency static electromagnetic fields, on a sarcoma cell line , were investigated.

Materials & Methods

Production of malignant (sarcoma) [MC] and smooth muscle cells (SMC). In this study, the malignant cells were isolated from selected sarcoma described tumors of Wistar rats. Fifteen (7 males and 8 females) Wistar rats, belonging to the fifth generation of a certain couple, 60 days old, were subcutaneously injected by 1 ml of 3,4-Benzopyrene solution (B[a]P) in Tricapryline at a final dose of 10,08 mgr/ml in their right scapula. . After 110 days (maximum 135 days), all the animals developed malignant tumors at the site of injection. All the tumors were histologicaly identified as leiomyosarcomas. The tumors were surgically removed and cut under aseptic conditions into pieces of 0.5cm size. Each pieces was placed immediately in cold Ringer's solution, then sliced down again to smaller pieces of 1 mm size and placed into 5 ml DMEM solution which contained small quantities of trypsin. The pieces in the solutions were kept at 37° C for 4 hours, with gentle mixing every15 minutes. Then they were centrifuged at 900 rpm for 10 minutes and the supernatant was rejected. The remained cells were resuspended in DMEM+10%FBS solution and seeded in plastic coated dishes of 52 mm size and subcultures of these cells were made, and were submitted to histological examination.

In order to verify if these cells are able to induce the same type of malignancy in rats, 4 million of these cells suspended in Hanks Salt solution were inoculated into every Wistar rat. The animals were anaesthetized with Midazolame and Ketamine, and surgical opening was made on the backside to their outer skin layer. The tissue underneath was traumatized by lancing with a sharp blade in order to bring fresh blood to the surface. Malignant cells were then aseptically infused into the operated area, closure of the open site was immediately performed. The animals developed

medium-sized malignant tumors (approximately 12 cm^3 size) within the first 10 days after inoculation.

Smooth muscle cells (SMC) were also isolated from the aorta of Wistar rats and subcultured by the methods described above.

Equipments used: Radio - frequency measurements and static electromagnetic field exposure of cells were performed by a device called MULTI CHANNEL DYNAMIC EXITER 100 V1 (MCDE) invented by K. Havelas and collaborators. The MCDE has been certified by the International Committee of Atomic Energy (E.K.E.F.E DEMOKRITOS, Athens Greece) for its safe use in humans and animals. This device consists of two basic parts: a) a diagnostic part with an EPR spectrometer's characteristics and b) an electromagnetic field generator of various intensities(from 1,1 to 1,11 +/-0.01 V/m for the electric field and 0.0027 to 0.0029+/-0.00005 A/m for the magnetic field) and radio – frequencies (from 1kHz to 1MHz) conducted by a sophisticated software. To use this software, first it is necessary to record the biological target system's frequencies and then, by using a specific algorithm , to calculate the appropriate electromagnetic frequencies that are needed , for the exposure of living target systems or cells (submitted for patent) .

Estimation of Malignant(MC) and Smooth Muscle Cells (SMC) electromagnetic radio - frequencies. A measurement of the MC and SMC biofrequencies was taken by the device described above, before their exposure to EMF. Electromagnetic radio frequency measurements were also taken from the survived MCs after their exposure to EMF for two consecutive days ..

Method of sarcoma cell exposure to EMF. Twelve Petri dishes with 10 ml growth medium each were seeded with the same number($1x10^5$) of sarcoma cells (time zero). The cells cultures were incubated in 37° C at 95% O_2 + 5% CO_2 for 48 hours and then the medium was changed. At 72 hours from time zero, six cell cultures (EMF cells) were placed into a Faraday apparatus at room temperature (RT) and exposed to electromagnetic radiofrequencies from 10 KHz to 120 KHz and intensities from 1,1 to 1,11 +/-0.01 V/m for the electric field and 0.0027 to 0.0029+/-0.00005 A/m for the magnetic field, for 45 minutes. The other six cell cultures (control cells) remained at RT for the same time as EMF cells without being exposed to EMF. The control and EMF cells were incubated once again at the same conditions as before for about seven hours. At 79h from zero time, the cells of each culture were counted, subcultured suspended at about $1x10^5$ cells per plate and incubated at the same as above conditions. The same procedure was repeated again as it is described above at 96hours from zero time. At 120hours the EMF cells were re-exposed again to the electromagnetic field as before and 24 hours after this exposure, both EMF and control cells in each plate were counted and examined microscopically. Then, to examine their proliferation rate in relation to time, both groups (EMF and control cells) were subcultured and incubated in order to estimate the time until confluence. The cells were then preserved in liquid nitrogen .

Sarcoma cell cycle determination. The preserved in liquid nitrogen EMF and control cells were defrost and subcultured until confluence. Twelve plates were then seeded with the same number of these sarcoma cells and incubated for 24 hours. The EMF cells were exposed to EMFs as before, after 24 h and 48 hours respectively. Six hours after the last session, samples from each plate were taken for testing in a

Bexton Dickinson flow cytometer. Cell samples were also tested for their ability to aggregate human platelets by an Aggregometer (CRONOLOG ,CA-500).

Estimation of platelet aggregation ability of sarcoma cells. The metastatic potential of sarcoma cells was determined from their ability to aggregate platelets before and after their exposure to electromagnetic fields. A total of 120 tests were performed on blood samples taken from six healthy volunteer donors , free from drugs or alcohol for about ten days before tests. The tests of platelets reactions were performed in platelet rich plasma (PRP) of the donors, prepared according to the manual of the apparatus. The CRONOLOG kit was used to verify the normal functional responses of platelets via the three aggregation pathways. Platelet activation and aggregation tests were performed after suspension in human PRPs of 500,000 of EMF-exposed or control cells .

Student's t-test was used for statistical evaluation of the results and $p<0.05$ was considered statistically significant.

Results

Cell proliferation rate. 24 hours after the first and second session of exposure to the electromagnetic fields, the proliferation rate of the sarcoma cells was slightly decreased, in comparison to those of the control (unexposed) cells ($p<0.05$). The microscopic examination also showed that the highest percentage of sarcoma cells was under stress (round-shaped cells with abortive pseudopodia and formation of nuclear membrane blebs). The multiplication rate of EMF-exposed cells was dramatically decreased at a percentage higher than 95%, ($p< 0,00001$ compared to the control) after 48 hours of incubation and most of the exposed malignant cells were found either dead (mainly apoptotic) or extremely stressed (round shaped cells, formation of bleeds in the outer cell membrane, absence of pseudopodia) (fig.1).

Also, the survived after EMF exposure, sarcoma cells showed a great difficulty in proliferating according to time till confluence (6 days incubation) in comparison the control cells (3 days incubation until confluence) (fig.2 and 3).

Malignant cells exposed for four repeated tumor cells sessions to the described above electromagnetic fields showed only a 20 % decrease of number of cells compared to the control sarcoma cells.

Sarcoma cell distribution in the cell cycle phases. Flow cytometry revealed that after the 4th exposure to EMF sessions 33% of cancer cells found to be in G0/G1, 9%in S phase, 2% in mitosis and 45% were undergoing apoptosis while the control cells found to be 36% in G0/G1, 38% in S phase, 19% in mitosis and 2% undergoing apoptosis (table 1).

Estimation of tumor cell "metastatic potential" (platelet aggregation ability). The aggregational ability of the control as well as of the exposed to EMF cells was 78%, and was almost equal to the aggregational ability of ADP (82%). According to the above EMF-exposure did not seem to affect significantly the "metastatic potential" of sarcoma cells(fig.4).

Estimation of cell electromagnetic radio - frequencies. Radio - frequencies of unexposed sarcoma cells were ranging between 10.5 to 120.5 KHz, of EMF-exposed sarcoma cells between 10 to 120 KHz and of the smooth muscle cells between 10 to 120 KHz. Spectrum analysis of the above estimations revealed that the radio-frequencies of sarcoma cells exposed to EMF showed significant differences compared to those of the unexposed sarcoma cells (control cells), presenting an almost 70% similarity to the radio-frequencies recorded from smooth muscle cells (data not shown).

Discussion

In the present study the actions of static electromagnetic fields , of low intensity (μT) at radiofrequencies, on (leiomyo)sarcoma cell lines obtained of B(a)P-treated Wistar rats, were investigated. These rat sarcoma cells have the following characteristics:: Sensitivity to antioxidant substances and free radical scavengers indicating that their proliferation is related to the release of reactive oxygen species (11,12,13),their proliferation can be inhibited via administration of COX-2 and 5-LOX inhibitors(14) and they possess the ability to induce (leiomyo)sarcomas when inoculated into Wistar rats.

The results of our experiments indicate that the application of radiofrequency EMFs according to electromagnetic resonance principles, can cause potent growth inhibition of (leiomyo)sarcoma cells (more than 95%).

Antiproliferative effects and significant morphological alterations on human melanoma cell lines have been achieved when cells exposed to low power millimeter waves in the 50-80 GHz frequency rate of the electromagnetic spectrum.(15).Apoptotic effects on human epidermoid cancer cells have also been induced by static electromagnetic fields of 1.95 GHz frequency(16).

In the present study antiproliferative and apoptotic effects have been achieved by exposing sarcoma cells to a static electromagnetic field of low energy waves and frequencies between 10 KHz to 120 KHz. This field is far lower in frequency and power than the upper limits of permitted exposure, being thus, safe for use in animals and humans (17).

From the literature, it is evident that the effect of EMFs are dependent on immediate interactions that affects the electronic spin of the atoms or molecules with uncoupled electrons in their external orbital, enhancing electron spin coupling and thus may help neutralization of free radicals, especially those produced by the activation of arachidonic acid cascades (14,16,18,19,20,21). It is also known that EMFs induce free radicals production that may act as activators of signal transduction pathways (10,19,20).

According to the above it is possible that the effects of these electromagnetic fields could be similar to the effects of antioxidants and free radical scavengers on sarcoma cell lines.

The high percentage of the exposed tumor cells found in apoptosis (45%) in comparison to that of the unexposed , control cells (2%), could be explained as the result of the EMFs effects on cellular membranes activating signal transduction

pathways leading to apoptic gene activation (5,7,16,22) or inactivation of anti-apoptotic genes(15). The low percentage of the cells found in synthesis and in mitosis (9 and 2% respectively) compared to that of the control cells (38 and 19% respectively) indicates that RF EMFs can act as cell cycle inhibitors , possibly to the effects of magnetic fields on DNA-synthesis (23,24,7). There is also evidence that exposure to electromagnetic fields may reduce immuno reactive p53 expression in tumor bearing mice (19), which has been found increased in BaP-induced sarcomas in Wistar rats (25). The lower sensitivity after the fourth exposure of the sarcoma cells to EMFs compared to their sensitivity after the first exposure indicates that the sarcoma cells may develop some type of resistance .

Our finding that the electromagnetic frequency pattern of the sarcoma cells changed after their exposure to EMF and resembled to that of smooth muscle cells, may possibly indicate that some type of sarcoma cell differentiation could take place.

The above is supported by our, yet unpublished data, indicating that these, EMF – treated, sarcoma cells fail to induce tumor development, when inoculated to Wistar rats in comparison to the unexposed sarcoma cells that induce tumors in 100% of the rats.

Nevertheless, the sarcoma cells exposed to EMF seems to retain their "metastatic potential" as they can still efficiently aggregate the platelets. There is also data that the EMFs, as well as the generalized electromagnetic radiation fields, are able to induce differentiation in cancer cells and other types of undifferentiated cells (26, 27).

It must be emphasized that for the first time a low intensity RFs EMF is used and it is essential this electromagnetic field to be carefully designed on the basis of the emitted electromagnetic frequencies from the target cells, in order to be effective. Also, the intensity of the electric field we used was 75 times lower and the intensity of the magnetic field was more than 1800 times less than the average of the international safety standards according to the International Committee of Atomic Energy (E.K.E.F.E DEMOKRITOS, Athens Greece) (17). Because of that, the use of this device as an electronic instrument in cancer treatment seems safe. Unpublished data of ours, from the follow up of tumor-bearing animals and cancer patients exposed to similar with the present investigation EMFs , are very encouraging.

Future targets

Our data concerning the alterations of radio-frequency pattern of sarcoma cells after repeated exposures to EMFs ,indicate that in order to affect the biological system of these cells, it is crucial to make readjustments of the RF, in order achieve a better electromagnetic resonance of cells , as close as possible as can be to that of the normal cells. If this hypothesis is right, it is expected that the final radio-frequencies of the sarcoma cells would be close to those of the smooth muscle cells. In this case the sarcoma cells should loose their malignant phenotype, so that their inoculation to Wistar rats will not cause malignant disease. Experiments going on , in our lab, seems to confirm this hypothesis.

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FIGURES

Fig.1: Sarcoma cells viability after exposure to EMF : 6 hours after exposure (p<0,01), 24 hours after exposure (p<0,009), 48 hours after exposure (p<0,00001). [SCw/oexEMF: Control (non exposed SC). SCwexEMF: Exposed SC].

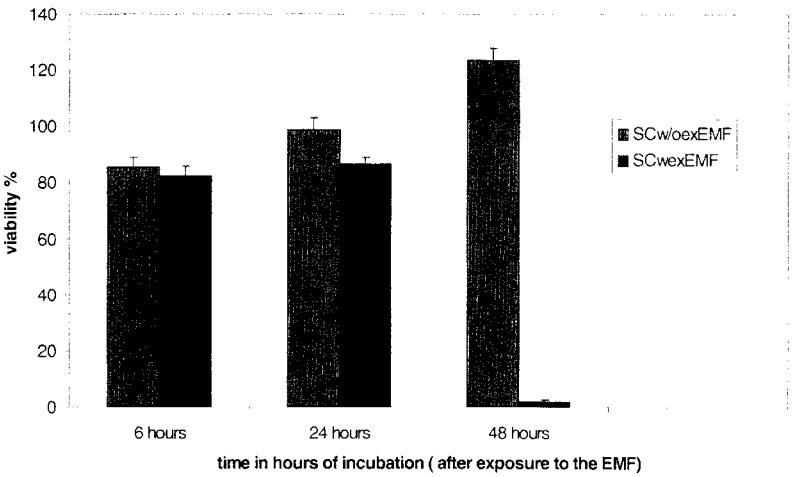


Fig 2. Proliferation rate of sarcoma cells survived after exposure to the EMF(note that the proliferation rate of these cells correspond to 20%, of the proliferation rate, after 72 hours of incubation, of unexposed sarcoma cells[control] , p < 0,001)

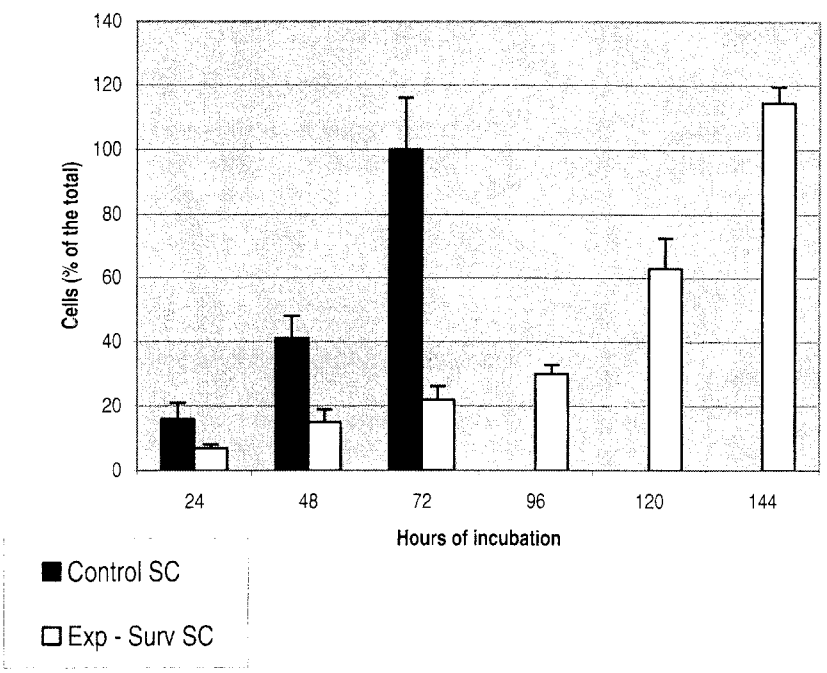


Fig.3 Viability of Smooth Muscle Cells (SMC) non exposed and exposed to the EMF. The non exposed SMC have a rate of growth 10% greater than the exposed ones (p<0,4)

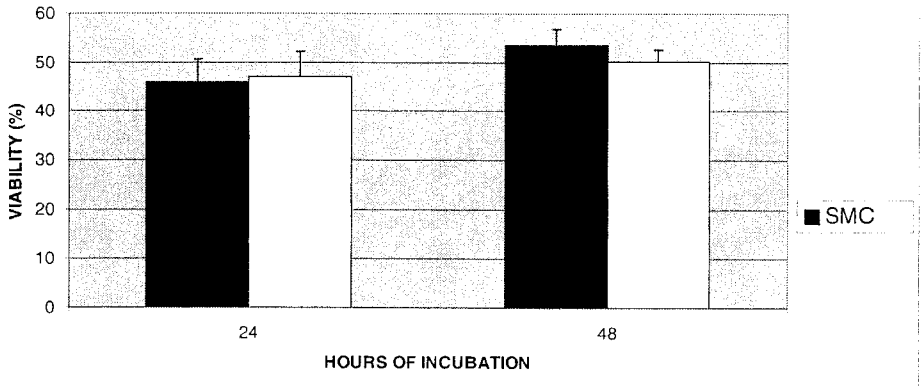
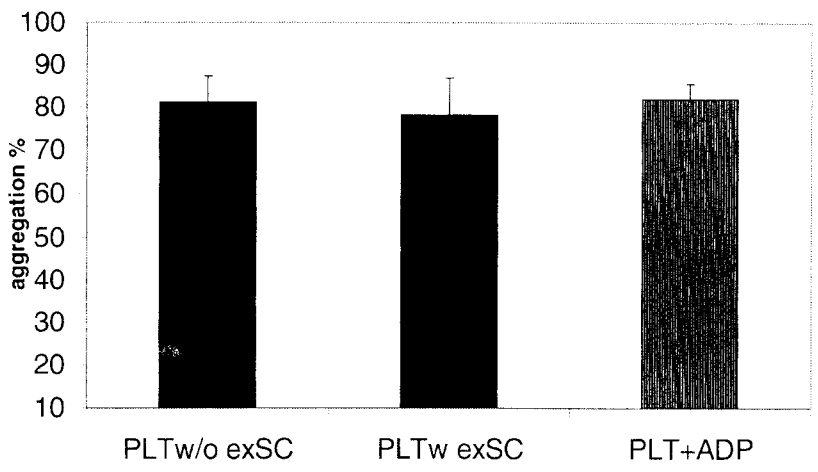


Fig.4: Platelet aggregation induced by exposed SM,non-exposed SM and ADP
(PLTw/oexSC:non-exposed SM,PLTw exSC:exposed SM)



TABLES

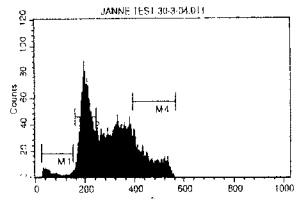
Table 1 : Flow cytometry for non exposed and exposed to EMF sarcoma cells .

The percentage of apoptotic nucleus in the group of control cells is very low (1,79 %) when the percentage of apoptotic nucleus in the group of exposed cells to EMF is much greater (45,34 %) .

Control (non exposed sarcoma cells)

Marker	Left	Right	Events	% Gated	Total
All	0	1023	9461	100,00	49,07
M1	26	149	169	1,79	0,88
M2	15	242	3366	35,58	17,46
M3	262	386	3549	37,51	18,41
M4	391	565	1774	18,75	9,20

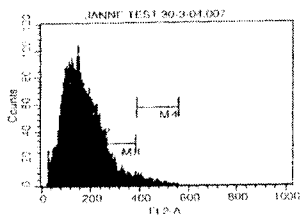
G0/G1 : 35,58%, S : 37,51% , G2/M : 18,75% ,
Apoptosis : 1,79%



Exposed sarcoma cells to EMF

Marker	Left	Right	Events	% Gated	Total
All	0	1023	13232	100,00	17,89
M1	26	149	5999	45,34	8,11
M2	159	242	4396	33,22	5,94
M3	262	386	1232	9,31	1,67
M4	391	565	217	1,64	0,29

G0/G1 : 33,22%, S : 9,31%, G2/M : 1,61% ,
Apoptosis : 45,34%



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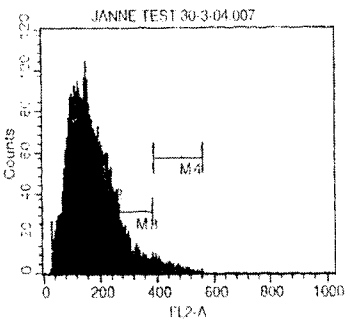

EFFECTS OF THE EXPOSURE OF SARCOMA CELL LINES TO LOW INTENSITY RADIOFREQUENCY STATIC ELECTRO-MAGNETIC FIELDS (EMFs) AND THEIR TUMORIGENICITY IN WISTAR RATS.

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Abstract

In this study we investigated the effects of low intensity static electromagnetic field (EMF) causing no thermal effects, on sarcoma cells, isolated from 3, 4-benzopyrene-induced (leiomyo)sarcomas in Wistar rats and their effects when inoculated in Wistar rats. The sarcoma cells were exposed to EMF using frequencies between 10 kHz to 120 kHz of the radiowave spectrum for 45 minutes. During a 24-hours period after cancer cell exposure to EMF, no inhibition of cell proliferation appeared. In contrast, at the end of 48 hours incubation time, the cancer cell proliferation was dramatically decreased in ratio > 95%. Also, the survived sarcoma cells after the exposure to EMF (2% of the total cell population exposed to EMF) showed a significant decrease to proliferate under the same culture conditions. These cells were then exposed once again to EMF for 45 minutes (totally 4 sessions of exposure) and tested using a flow cytometer. It was found that a great percentage (45%) of these cells, double exposed to EMF, was apoptotic and only a small percentage of them was found under mitosis (2 %). Additionally, the cells were counted and tested using an aggregometer for their ability to aggregate the platelets (an indicator of their metastatic potential) and they didn't show any difference in comparison to the sarcoma cells not exposed to EMF (control cells). Both, exposed and unexposed cells induced soft tissue tumors, when inoculated in Wistar rats, but mean survival time was significantly prolonged and tumor growth rate significantly reduced in the group inoculated by exposed to EMF sarcoma cells(Experimental group),in comparison to the non-exposed group(control group). Lung metastases were also reduced in the experimental group compared to the control group. Results

indicate that surviving to EMF-exposure sarcoma cells, manifest a milder malignant phenotype when inoculated to animals in comparison to the non-exposed cells. sarcoma cells sarcoma cells

Key Words: Sarcoma cells, Static electromagnetic fields, radiofrequency waves , resonance, platelet aggregation, metastasis, Wistar rats

Abbreviations : Electromagnetic fields : EMF , Smooth Muscle cells : SMC, Malignant sarcoma cells : MC, SC, Platelets Reach Plasma : PRP , 3,4-benzopyrene : B[a]P.: DMEM , Fetal Bovine serum : FBS , Radio – frequencies : RF, Electron Paramagnetic Resonance : EPR , Electron Spin Resonance : ESR.

Introduction

There is a lot of data dealing with the effects of electromagnetic fields (EMFs) on cells, experimental animals and humans, some of them referred to application of electromagnetic resonance principles(1, 2). Dealing with malignancy , the following main concepts have been expressed, so far, depending on the intensity, frequency and duration of application of the electromagnetic waves : The EMFs may act as co-carcinogens in combination with the initiating carcinogen , especially in experimental animals and the EMFs can exert anticarcinogenic effects, inhibiting the proliferation of malignant cells in vitro as well as decreasing the size of the experimental tumors in vivo (3,4,5,6). The studies on EMFs pro-carcinogenic effects in experimental animals are however, not numerous and it seems that the described methods have a lot of uncertainty (3,4). In comparison, the studies on EMF anticancer effects are abundant and their methodology is well documented (7). It has been also shown, that the

cytostatic effects of the EMFs on cancer cells are not related to their thermal effects but are exerted via temperature-independent actions(8,9,10).

In the present study the effects exerted by low intensity radiofrequency static electromagnetic fields, on a sarcoma cell line , its tumorigenic effects in Wistar rats, were investigated.

Materials & Methods

Production of malignant (sarcoma) [MC] and smooth muscle cells (SMC). In this study, the malignant cells were isolated from selected sarcoma described tumors of Wistar rats. Fifteen (7 males and 8 females) Wistar rats, belonging to the fifth generation of a certain couple, 60 days old, were subcutaneously injected by 1 ml of 3,4-Benzopyrene solution (B[a]P) in Tricapryline at a final dose of 10,08 mgr/ml in their right scapula. . After 110 days (maximum 135 days), all the animals developed malignant tumors at the site of injection. All the tumors were histologicaly identified as leiomyosarcomas. The tumors were surgically removed and cut under aseptic conditions into pieces of 0.5cm size. Each pieces was placed immediately in cold Ringer's solution, then sliced down again to smaller pieces of 1 mm size and placed into 5 ml DMEM solution which contained small quantities of trypsin. The pieces in the solutions were kept at 37° C for 4 hours, with gentle mixing every15 minutes. Then they were centrifuged at 900 rpm for 10 minutes and the supernatant was rejected. The remained cells were resuspended in DMEM+10%FBS solution and seeded in plastic coated dishes of 52 mm size and subcultures of these cells were made, and were submitted to histological examination.

In order to verify if these cells are able to induce the same type of malignancy in rats, 4 million of these cells suspended in Hanks Salt solution were inoculated into every Wistar rat. The animals were anaesthetized with Midazolame and Ketamine, and surgical opening was made on the backside to their outer skin layer. The tissue underneath was traumatized by lancing with a sharp blade in order to bring fresh blood to the surface. Malignant cells were then aseptically infused into the operated area, closure of the open site was immediately performed. The animals developed medium-sized malignant tumors (approximately 12 cm^3 size) within the first 10 days after inoculation.

Smooth muscle cells (SMC) were also isolated from the aorta of Wistar rats and subcultured by the methods described above.

Equipments used: Radio - frequency measurements and static electromagnetic field exposure of cells were performed by a device called MULTI CHANNEL DYNAMIC EXITER 100 V1 (MCDE) invented by K. Havelas and collaborators. The MCDE has been certified by the International Committee of Atomic Energy (E.K.E.F.E DEMOKRITOS, Athens Greece) for its safe use in humans and animals. This device consists of two basic parts: a) a diagnostic part with an EPR spectrometer's characteristics and b) an electromagnetic field generator of various intensities(from 1,1 to 1,11 +/-0.01 V/m for the electric field and 0.0027 to 0.0029+/-0.00005 A/m for the magnetic field) and radio – frequencies (from 1kHz to 1MHz) conducted by a sophisticated software. To use this software, first it is necessary to record the biological target system's frequencies and then, by using a specific algorithm , to calculate the appropriate electromagnetic frequencies that are needed , for the exposure of living target systems or cells (submitted for patent) .

Sarcoma cell cycle determination. The preserved in liquid nitrogen EMF and control cells were defrost and subcultured until confluence. Twelve plates were then seeded with the same number of these sarcoma cells and incubated for 24 hours. The EMF cells were exposed to EMFs as before, after 24 h and 48 hours respectively. Six hours after the last session, samples from each plate were taken for testing in a Bexton Dickinson flow cytometer. Cell samples were also tested for their ability to aggregate human platelets by an Aggregometer (CRONOLOG ,CA-500).

Estimation of platelet aggregation ability of sarcoma cells. The "metastatic potential" of sarcoma cells was determined from their ability to aggregate platelets before and after their exposure to electromagnetic fields. A total of 120 tests were performed in blood samples withdrawn from six healthy volunteer donors , free from drugs or alcohol for about ten days before tests. The tests on platelet aggregation were performed in platelet rich plasma (PRP) of the donors, prepared according to the manual of the apparatus. The CRONOLOG kit was used to verify the normal functional responses of platelets via the three aggregation pathways. Platelet activation and aggregation tests were performed after suspension in human PRPs of 500,000 of EMF-exposed or unexposed sarcoma cells .

Animal studies: Two groups of male Wistar rats , 3 months old, 250+/- 15 g, b.w, were used in order to estimate the malignant potncy of unexposed and exposed sarcoma cells, in vivo, as follows:

a). **Control Group (CG)** : consisted of 10 Wistar rats which were inoculated by $4X10^6$ unexposed to EMF sarcoma cells,each. .

b). **Experimental Group (EG):** 10 Wistar rats were inoculated by $4X10^6$ sarcoma cells exposed to the previously described EMF.

-

Inoculation of cells in Wistar rats: $4X10^6$of sarcoma cells (exposed or unexposed to EMF) were suspended in 1 ml of Hank's solution. The animals were anesthetized with ketamine (3 mg and 3.5 mg /body weight from each drug) and a surgical opening was made to their outer skin layer of their dorsal area (by the right scapula) deep up to the muscle layer . The tissue underneath was then traumatized by lancing with a sharp blade (five parallel cuts of 5mm length) till the production of the slight hemorrhage. Sarcoma cells (exposed or unexposed) at a number of $4X10^6$ cells, were then aseptically infused into the operated area and closure of the open sites was immediately performed.

Animals of both groups were then placed into cages (2 animals in each cage) and kept at temperature of 19 ^{o} C ± 1,2 ^{o} C, in 12 hours light and 12 hours dark Animals feeding and water drinking was ad libidum. Dead animals of all groups were autopsied, tumor were carefully excised, weighed and tumor or muscles at the site of cell inoculation removed for histology. Histology for possible metastases was also performed in lungs , stomach, intestine and kidneys in all animals of both groups.

.In both groups mean survival time of animals(MST -days) was calculated, as well as mean tumor weight (MTW) and mean tumor growth rate (MTGR) as a ratio of :

$$\text{tumor growth rate (TGR)} = \frac{\text{tumor weight (grams)}}{\text{survival time (days)}} \text{ (g/d), in each animal.}$$

Time till tumor appearance (TTA-days) as a palpable mass after cell inoculation , was also estimated.

Student's t-test was used for statistical evaluation of the results and $p<0.05$ was considered statistically significant.

Results

Cell proliferation rate. 24 hours after the first and second session of exposure to the electromagnetic fields, the proliferation rate of the sarcoma cells was slightly decreased, in comparison to those of the control (unexposed) cells ($p<0.05$). The microscopic examination also showed that the highest percentage of sarcoma cells was under stress (round-shaped cells with abortive pseudopodia and formation of nuclear membrane blebs). The multiplication rate of EMF-exposed cells was dramatically decreased at a percentage higher than 95%, ($p< 0,00001$ compared to the control) after 48 hours of incubation and most of the exposed malignant cells were found either dead (mainly apoptotic) or extremely stressed (round shaped cells, formation of bleeds in the outer cell membrane, absence of pseudopodia) (fig.1).

Also, the survived after EMF exposure, sarcoma cells showed a great difficulty in proliferating according to time till confluence (6 days incubation) in comparison the control cells (3 days incubation until confluence) (fig.2 and 3).

Malignant cells exposed for four repeated tumor cells sessions to the described above electromagnetic fields showed only a 20 % decrease of number of cells compared to the control sarcoma cells.

Sarcoma cell distribution in the cell cycle phases. Flow cytometry revealed that after the 4th exposure to EMF sessions 33% of cancer cells found to be in G0/G1, 9%in S phase, 2% in mitosis and 45% were undergoing apoptosis while the control cells found to be 36% in G0/G1, 38% in S phase, 19% in mitosis and 2% undergoing apoptosis (table 1).

Estimation of tumor cell "metastatic potential" (platelet aggregation ability). The induction of aggregation of human PRP, of the unexposed sarcoma as well as of the exposed to EMF cells was 78%, and was almost equal to the aggregation ability of ADP (82%). According to the above EMF-exposure did not seem to affect significantly the "metastatic potential" of sarcoma cells (fig.4).

Estimation of cell electromagnetic radio - frequencies. Radio - frequencies of unexposed sarcoma cells were ranging between 10.5 to 120.5 KHz, of EMF-exposed sarcoma cells between 10 to 120 KHz and of the smooth muscle cells between 10 to 120 KHz. Spectrum analysis of the above estimations revealed that the radio-frequencies of sarcoma cells exposed to EMF showed significant differences compared to those of the unexposed sarcoma cells (control cells), presenting an almost 70% similarity to the radio-frequencies recorded from smooth muscle cells (data not shown).

Tumor induction in Wistar rats: Our results according to the different groups of the study are as follows :

Animals of the control group (CG), inoculated with non –exposed to EMF sarcoma cells, developed tumors at the site of inoculation, which were histologically identified as spindle cells sarcomas. Mean survival time (MST) of the tumor bearing animals of this group was 22 ± 4,9 days after inoculation, while mean tumor weight was 84,9 ± 22.1 gr and the median tumor growth rate (TGR) was 3.85 ± 0.9 gr/day.

On the other hand in the experimental group (EG) tumor induction was also 100%, but mean survival time was 46±17,9 days. The median weight of tumors was 89,7± 27,7 gr and median tumor growth rate 1,9 ± 0,9 gr/day. The histological type of all tumors was spindle cells sarcomas. Moreover 7 out of 10 animals (70%) of the CG manifested lung metastases in contrast to 4 out 10 animals (40%), which possessed lung metastases. Statistical evaluation revealed a statistically significant difference in mean survival time and tumor growth rate between the two groups and a not significant difference in mean tumor weights (table 1).

Moreover at the 30th after cell inoculation) none of the animals of the control group(CG) was surviving (the first animal died at the 14th day and the last in the30th day), while 8 .animals out 10 of the experimental group were still alive and in good condition in the 30th day and the last one died in the 72th day after cell inoculation (see also death rate curve fig.....).

Table 1:

	CONTROL GROUP	EXPERIMENTAL GROUP
MST (days)	22 ± 4,9*	46,5 ±17,9*
MTW(grams)	84,9 ± 22,1	89,7± 27,7

TGR (gr/day)	3,85 ± 0,9**	1,9 ± 0,9**

Results statistically significant (p<0.0....,& p<0.0...)

Discussion

In the present study the actions of static electromagnetic fields , of low intensity (μT) at radiofrequencies, on (leiomyo)sarcoma cell lines obtained from B(a)P-treated Wistar rats,and their carcinogenicity when inoculated in Wistar rats were investigated. These rat sarcoma cells have the following characteristics: Sensitivity to antioxidant substances and free radical scavengers indicating that their proliferation is related to the release of reactive oxygen species (11,12,13),their proliferation can be inhibited via administration of COX-2 and 5-LOX inhibitors(14) and they possess the ability to induce (leiomyo)sarcomas when inoculated into Wistar rats.

The results of our experiments indicate that the application of radiofrequency EMFs according to electromagnetic resonance principles, can cause potent growth inhibition of (leiomyo)sarcoma cells (more than 95%).

Antiproliferative effects and significant morphological alterations on human melanoma cell lines have been achieved when cells exposed to low power millimeter waves in the 50-80 GHz frequency rate of the electromagnetic

spectrum.(15).Apoptotic effects on human epidermoid cancer cells have also been induced by static electromagnetic fields of 1.95 GHz frequency(16).

In the present study antiproliferative and apoptotic effects have been achieved by exposing sarcoma cells to a static electromagnetic field of low energy waves and frequencies between 10 KHz to 120 KHz. This field is far lower in frequency and power than the upper limits of permitted exposure, being thus, safe for use in animals and humans (17).

From the literature, it is evident that the effect of EMFs are dependent on immediate interactions that affects the electronic spin of the atoms or molecules with uncoupled electrons in their external orbital, enhancing electron spin coupling and thus may help neutralization of free radicals, especially those produced by the activation of arachidonic acid cascades (14,16,18,19,20,21). It is also known that EMFs induce free radicals production that may act as activators of signal transduction pathways (10,19,20).

According to the above it is possible that the effects of these electromagnetic fields could be similar to the effects of antioxidants and free radical scavengers on sarcoma cell lines.

The high percentage of the exposed tumor cells found in apoptosis (45%) in comparison to that of the unexposed , control cells (2%), could be explained as the result of the EMFs effects on cellular membranes activating signal transduction pathways leading to apoptic gene activation (5,7,16,22) or inactivation of anti-apoptotic genes(15). The low percentage of the cells found in synthesis and in mitosis (9 and 2% respectively) compared to that of the control cells (38 and 19% respectively) indicates that RF EMFs can act as cell cycle inhibitors , possibly to the effects of magnetic fields on DNA-synthesis (23,24,25). There is also evidence that

exposure to electromagnetic fields may reduce immuno reactive p53 expression in tumor bearing mice (19), which has been found increased in BaP-induced sarcomas in Wistar rats (26). The lower sensitivity after the fourth exposure of the sarcoma cells to EMFs compared to their sensitivity after the first exposure indicates that the sarcoma cells may develop some type of resistance .

Our finding that the electromagnetic frequency pattern of the sarcoma cells changed after their exposure to EMF and resembled to that of smooth muscle cells, may possibly indicate that some type of sarcoma cell differentiation could take place.

The above is supported by results obtained by the animal groups inoculated with EMF-exposed and non-exposed sarcoma cells. Our data, indicate that although EMF –exposed, and non-exposed sarcoma cells induce tumor development, when inoculated to Wistar rats in 100% of the animals, there is a significant prolongation of the survival time of the animals bearing tumors due to inoculation of EMF-exposed sarcoma cells(experimental group) in comparison to those bearing tumors from non-exposed sarcoma cells (control group- $p< 0.0...$). Similar results were observed in tumor growth rate between the two groups. There is also a significant reduction in lung metastases in the experimental group (EG) compared to the control group(CG), [in 40% and 70% of the animals ,correspondingly]. The above indicate that EMF – exposed sarcoma cells manifest a milder malignat phenotype when inoculated to animals, in comparison to non-exposed sarcoma cells. According to our knowledge, anticarcinogenic effects of EMFs on animal models have also been investigated (7,) , but this is the first experiment investigating the in vivo properties of EMF-treated malignant cells , in animal models.

Nevertheless, the sarcoma cells exposed to EMF seems to retain in a way their "metastatic potential" as they can still efficiently aggregate the platelets, but in vivo

their "metastatic potency" seems to be reduced, as it is evident from our results on Wistar rats. There is also data that the EMFs, as well as the generalized electromagnetic radiation fields, are able to induce differentiation in cancer cells and other types of undifferentiated cells (27,28).

It must be emphasized that for the first time a low intensity RFs EMF is used and it is essential this electromagnetic field to be carefully designed on the basis of the emitted electromagnetic frequencies from the target cells, in order to be effective. Also, the intensity of the electric field we used was 75 times lower and the intensity of the magnetic field was more than 1800 times less than the average of the international safety standards according to the International Committee of Atomic Energy (E.K.E.F.E DEMOKRITOS, Athens Greece) (17). Because of that, the use of this device as an electronic instrument in cancer treatment seems safe. Unpublished data of ours, from the follow up of tumor-bearing animals and cancer patients exposed to similar with the present investigation EMFs , are very encouraging.

Future targets

Our data concerning the alterations of radio-frequency pattern of sarcoma cells after repeated exposures to EMFs ,indicate that in order to affect the biological system of these cells, it is crucial to make readjustments of the RF, in order achieve a better electromagnetic resonance of cells , as close as possible as can be to that of the normal cells. If this hypothesis is right, it is expected that the final radio-frequencies of the sarcoma cells would be close to those of the smooth muscle cells. In this case the sarcoma cells should loose their malignant phenotype, so that their inoculation to

Wistar rats will not cause malignant disease. Experiments going on , in our lab, seems to confirm this hypothesis.

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survival, tumor growth inhibition and decreased immunoreactive p53 after exposure to magnetic fields. e. Bioelectromagnetics 2002; **23**:230-238 (Υπάρχει και ως 7)

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FIGURES

Fig.1: Sarcoma cells viability after exposure to EMF : 6 hours after exposure ($p<0,01$), 24 hours after exposure ($p<0,009$), 48 hours after exposure ($p<0,00001$). [SCw/oexEMF: Control (non exposed SC). SCwexEMF: Exposed SC].

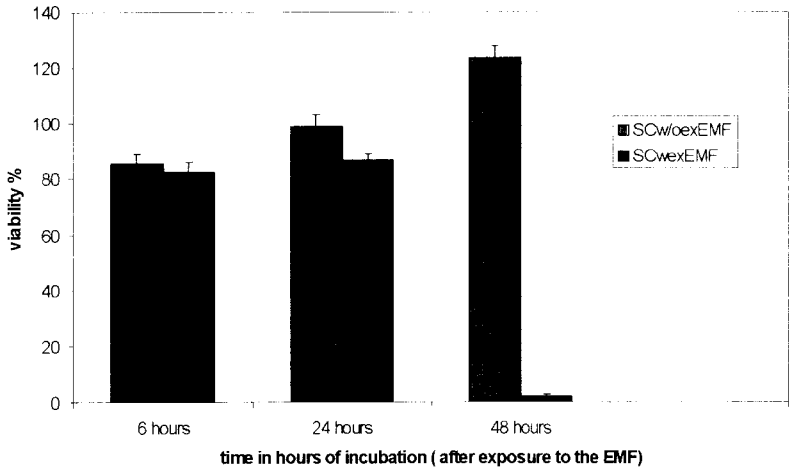


Fig 2. Proliferation rate of sarcoma cells survived after exposure to the EMF(note that the proliferation rate of these cells correspond to 20%, of the proliferation rate, after 72 hours of incubation, of unexposed sarcoma cells[control] , $p < 0,001$)

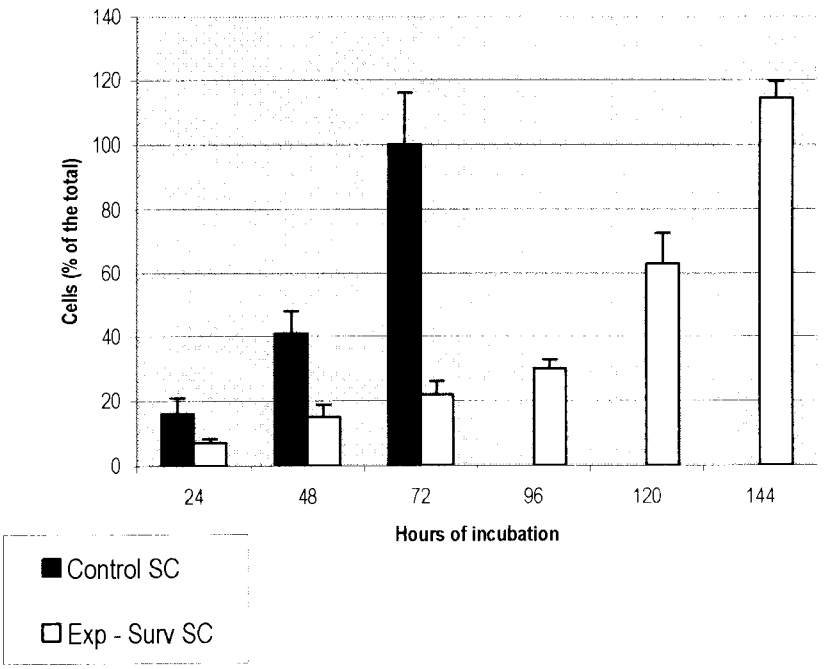


Fig.3 Viability of Smooth Muscle Cells (SMC) non exposed and exposed to the EMF. The non exposed SMC have a rate of growth 10% greater than the exposed ones (p<0,4)

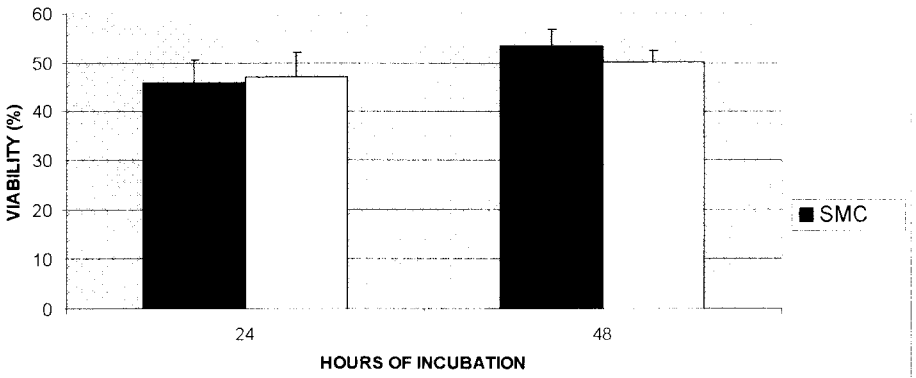


Fig.4: Platelet aggregation induced by exposed SM,non-exposed SM and ADP
(PLTw/oexSC:non-exposed SM,PLTw exSC:exposed SM)

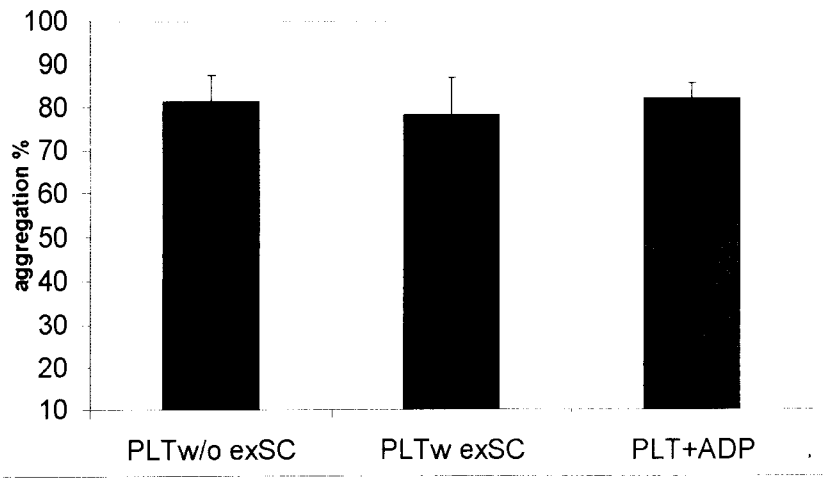


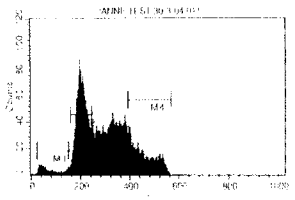
Table 1 : Flow cytometry for non exposed and exposed to EMF sarcoma cells .

The percentage of apoptotic nucleus in the group of control cells is very low (1,79 %) when the percentage of apoptotic nucleus in the group of exposed cells to EMF is much greater (45,34 %) .

Control (non exposed sarcoma cells)

Marker	Left	Right	Events	% Gated	Total
All	0	1023	9461	100,00	49,07
M1	26	149	169	1,79	0,88
M2	15	242	3366	35,58	17,46
M3	262	386	35,49	37,51	18,41
M4	391	565	1774	18,75	9,20

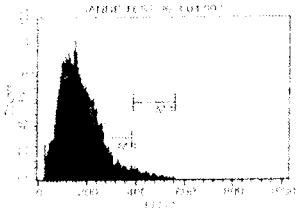
G0/G1 : 35,58%, S : 37,51% , G2/M : 18,75% ,
Apoptosis : 1,79%



Exposed sarcoma cells to EMF

Marker	Left	Right	Events	% Gated	Total
All	0	1023	13232	100,00	17,89
M1	26	149	5999	45,34	8,11
M2	159	242	4396	33,22	5,94
M3	262	386	1232	9,31	1,67
M4	391	565	217	1,64	0,29

G0/G1 : 33,22%, S : 9,31%, G2/M : 1,61% ,
Apoptosis : 45,34%



Exposed sarcoma cells to EMF

Marker	Left	Right	Events	% Gated	Total
All	0	1023	13232	100,00	17,89
M1	26	149	5999	45,34	8,11
M2	159	242	4396	33,22	5,94
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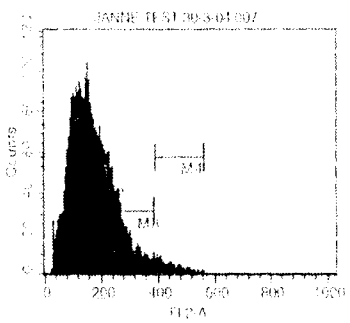
Apoptosis : 45,34%



HELLENIC PETROLEUM S.A.

ELEFSIS REFINERY

CERTIFICATE OF QUALITY
LABORATORY REPORT

PRODUCT: JET A1

PROPERTIES		RESULTS	METHOD ASTM/IP
Density at 15 °C	Kg/m^3	799.3	D-1298
Appearance (visual)		Clear bright	
Colour		21	D-156
Total acidity	mgKOH/g	0.004	IP-354
Aromatics	% vol	17.0	D-1319
Sulfur total	% wt	0.27	D-4294
Sulfur, mercaptan or Doctor test	% wt	0.0012	D-3277 D-4952
Hydroprocessed component in batch	% vol	nil	
Severly hydroprocessed components	% vol	nil	
Distillation	°C		D-86
I.B.P.	°C	154	
10 % vol	°C	175	
20 % vol	°C	181	
50 % vol	°C	200	
90 % vol	°C	236	
End point	°C	252	
Loss	% vol	0.8	
Residue	% vol	0.5	
Flash point (Tag)	°C	43	D-56
Freezing point	°C	-49	D-2386

PROPERTIES		RESULTS	METHOD ASTM/IP
Viscosity at -20 °C	cSt		D-445
Specific Energy Net	MJ/Kg		D-4529
Water reaction		1B/2	D-1094
Smoke point	mm		D-1322
Corrosion, copper (2h at 100 °C)		1a	D-130
Thermal stability(JFTOT) (control temperature 260 °C)			D-3241
a-Filter pressure differential	mm Hg	Zero	
b-Tube Deposit Rating (visual)		Zero,No peacock or abnormal colour deposits	
Existent Gum	mg/100ml	1.5	D-381
Particulate Contamination	mg/l	0.40	D-5452
WSIM(Microseparometer, rating) with Static dissipator additive without Static dissipator additive		93	D-3948
Electrical conductivity	Ps/m	5	D-2624
Lubricity Bocle wear scar diameter	mm		D-5001
Antioxidant in hydroprocessed & synthetic fuels	mg/l		
in non-hydroprocessed fuels	mg/l		
Metal deactivator	mg/l		
Static dissipator	mg/l		
First doping Stadis 450	mg/l		
Re-doping	mg/l		

This product meets joint fuelling system check list for Jet A-1
latest issue (20) -March 2005'.
which embodies the following Specifications.
a-British MoD DEF STAN 91-91/Issue 5, dated 8 February 2005, Jet A-1.
b-ASTM D-1655-04a, Jet A-1.

CHIEF CHEMIST:

HELLENIC PETROLEUM S.A.
ELEFSIS REFINERY

CERTIFICATE OF QUALITY
LABORATORY REPORT

PRODUCT: **UNLEADED GASOLINE 95 RON**

PROPERTIES		RESULTS	METHOD
DENSITY at 15 °C	Kg/m^3	743.0	EN ISO 12185
APPEARANCE		CLEAR BRIGHT	
COLOUR		UNDYED	
DISTILLATION F.B.P.	deg C	194	prEN ISO 3405
RESIDUE	% vol	1.0	prEN ISO 3405
RECOVERED at 70 °C	% vol	32	prEN ISO 3405
RECOVERED at 100 °C	% vol	55	prEN ISO 3405
RECOVERED at 150 °C	% vol	84.5	prEN ISO 3405
REID VAPOR PRESSURE AT 100°F	kpa	79	prEN 13016
SULFUR	mg/Kg	39	EN ISO 8754
VOLATILITY INDEX (VLI)			ISO 3405,EN 12
PHOSPHORUS		NIL	ASTM - D 2599
ETHERS (5 or more C-atoms)	% vol	4.5	EN 1601
TOTAL ACIDITY	mg/KOH/g	NIL	ASTM - D 974
COPPER CORROSION		1A	EN ISO 2160
LEAD	mgr/Kg	2.0	EN 237
RON		96.6	EN 25164
MON		85.0	EN 25163
OXIDATION STABILITY	min	>360	ISO 7536
EXISTENT GUMS (WASHED)	mg/100ml	3	EN ISO 6246
AROMATICS	% vol	31.9	EN 12177
OLEFINS	% vol	13.2	EN 12177
BENZENE	% vol	0.9	EN 12177
TOTAL OXYGEN	%wt	0.8	prEN 13132
QUINIZARINE	mg/l	3.0	

CHIEF CHEMIST:

CERTIFICATE OF QUALITY
LABORATORY REPORT

HELLENIC PETROLEUM S.A.

ELEFSIS REFINERY

PRODUCT: DIESEL AUTOMOTIVE

PROPERTIES		RESULTS	METHOD
DENSITY at 15 °C	Kg/m^3	834.9	EN ISO 12185
COLOUR		0.5	ASTM D1500
APPEARANCE		CLEAR BRIGHT	
DISTILLATION I.B.P.	deg C		prEN ISO 3405/98
DISTILLATION 5%	deg C		
DISTILLATION 10%	deg C		
DISTILLATION 20%	deg C		
DISTILLATION 50%	deg C		
DISTILLATION 65%	deg C	294	
DISTILLATION 85%	deg C	320	
DISTILLATION 90%	deg C		
DISTILLATION 95%	deg C	344	
DISTILLATION F.B.P.	deg C		
FLASH POINT	deg C	82	EN 22719
SULFUR	ppm	45	EN ISO 8754/95
COPPER CORROSION		1A	EN ISO 2160
C.F.P.P.	deg C	-10	EN-116
VISCOSITY KINEMATIC at 40°C	cst	3.2	EN ISO 3104
POUR POINT	deg C		ISO 3016
CETANE INDEX		55.2	EN ISO 4264
CETANE NUMBER		55.5	EN ISO 5165
CARBON CONRADSON RESIDUE	% wt	0.01	EN ISO 10370
ASH	% wt	0.003	EN ISO 6245
LUBRICITY (wsd1.4) at 60 °C	µm	190	ISO 12156-1
OXIDATION STABILITY	g/m^3	10	EN ISO 12205
SUSPENDED MATTER	g/m^3	8	EN 12662
FAME content	%v/v	2.0	EN 14078
WATER	ppm	140	prEN ISO 12937/96
POLYCYCLIC AROMATIC HYDROCARBONS	% wt	4.2	IP - 391

CHIEF CHEMIST:

CERTIFICATE OF QUALITY
LABORATORY REPORT

HELLENIC PETROLEUM S.A.

ELEFSIS REFINERY

PRODUCT: DIESEL HEATING

PROPERTIES		RESULTS	METHOD
DENSITY at 15 °C	Kg/m^3	842.7	EN ISO 12185
COLOUR		3.5	ASTM D1500
SOLVENT YELLOW 124	mg/kg	6.0	
DISTILLATION I.B.P.	deg C		prEN ISO 3405/98
DISTILLATION 5%	deg C		
DISTILLATION 10%	deg C		
DISTILLATION 20%	deg C		
DISTILLATION 50%	deg C		
DISTILLATION 65%	deg C		
DISTILLATION 85%	deg C	346	
DISTILLATION 90%	deg C		
DISTILLATION 95%	deg C		
DISTILLATION F.B.P.	deg C		
FLASH POINT	deg C	65	EN 22719
SULFUR	%wt	0.19	EN ISO 8754/95
COPPER CORROSION		1A	EN ISO 2160
C.F.P.P.	deg C	-5	EN-116
VISCOSITY KINEMATIC at 40°C	cst	3.1	EN ISO 3104
POUR POINT	deg C	-21	ISO 3016
CETANE INDEX		52.0	EN ISO 4264
CETANE NUMBER			EN ISO 5165
CARBON CONRADSON RESIDUE	% wt	<0.1	EN ISO 10370
ASH	% wt	<0.01	EN ISO 6245
LUBRICITY (wsd1.4) at 60 °C	µm		ISO 12156-1
OXIDATION STABILITY	g/m^3		EN ISO 12205
SUSPENDED MATTER	g/m^3		EN 12662
FAME content	%v/v		EN 14078
WATER	ppm		prEN ISO 12937/96
POLYCYCLIC AROMATIC HYDROCARBONS	% wt		IP - 391

CHIEF CHEMIST:

(15.2) TABLE
Specifications of Gasoline

a/a	Type of Gasoline Parameters	95 RON Limits	100 RON Limits	98 RON Limits	96 RON LRP Limits
1.	Density at 15, ^{O}C, kg/m^3	720-775	720-790 max.	720-790 max.	720-775
2	Vapor Pressure Kpa(psi) max -Κλάση A:απο 1/5 εως 30/9 -Κλάση B:απο 1/11 εως 31/3 - Κλάση C1: 1/4 -30/4 & 1/10- 31/10	45(6.2)-60(8.7) 50(6.7)-80(11.6) 50(6.7)-80(11.6)	45(6.2)-60(8.7) 50(6.7)-80(11.6) 50(6.7)-80(11.6)	45(6.2)-60(8.7) 50(6.7)-80(11.6) 50(6.7)-80(11.6)	45(6.2)-60(8.7) 50(6.7)-80(11.6) 50(6.7)-80(11.6)
3.	Distillation a) Recovered at 70 ^{O}C, %vol - Κλάση A:απο 1/5 εως 30/9 - Κλάση B:απο 1/11 εως 31/3 - Κλάση C1: 1/4 -30/4 & 1/10- 31/10	 20-48 22-50 22-50	 10-48 10-50 10-50	 10-48 10-50 10-50	 20-48 22-50 22-50
	b) Recovered at 100 ^{O}C, vol% min - Κλάση A:απο 1/5 εως 30/9 - Κλάση B:απο 1/11 εως 31/3 - Κλάση C1: 1/4 -30/4 & 1/10- 31/10	 46-71 46-71 46-71	 46-71 46-71 46-71	 46-71 46-71 46-71	 46-71 46-71 46-71
	c) Recovered at 150 ^{O}C, vol% min. - Κλάση A:απο 1/5 εως 30/9 - Κλάση B:απο 1/11 εως 31/3 - Κλάση C1: 1/4 -30/4 & 1/10- 31/10	 75 75 75	 75 75 75	 75 75 75	 75 75 75
	e) Final Boiling point, ^{O}C, max	210	210	210	210
	f) Distillation residue, %vol, max	2	2	2	2
4.	Volatility Index (VLI), max - Κλάση A:απο 1/5 εως 30/9 - Κλάση B:απο 1/11 εως 31/3 - Κλάση C1: 1/4 -30/4 & 1/10- 31/10	 - - 1050	 - - 1050	 - - 1050	 - - 1050
5.	Sulfur, mg/kg, max	50	50	50	50
6.	Lead g/l max	0.005	0.005	0.005	0.005
7.	Octane number RON/MON, min	95/85	100/87.5	98/86	96/85
8.	Copper corrosion ASTM No, max (3hrs at 50 ^{O}C)	1	1	1	1
9.	Oxidation stability, minutes, min	360	360	360	360
10	Existent gums, mg/100ml, max (washed)	5	5	5	5
11.	Benzene, %vol, max	1.0	1.0	1.0	1.0
12.	Aromatics, %vol	35.0	35.0	35.0	35.0
13.	Olefins, %vol	18.0	18.0	18.0	18.0
14.	Phosphorus	(c) Ø	(c) Ø	(c) Ø	
15	Oxygenates Total Oxygen, wt%, max	2.7 3	2.7 Ø	2.7 Ø	2.7 Ø
16.	Quinizarine mg/l				10 - 20
17.	Potassium mg/kg				green
18.	Colour	undyed	undyed	undyed	
19	Oxygenates content: % vol. max. Methanol Ethanol Iso-propyl alcohol Eso-butyl alcohol Oert-butyl alcohol Ethers (5 or more C-atoms) Ither-oxygenates	 3 5 10 10 7 15 10	 3 5 10 10 7 15 10	 3 5 10 10 7 15 10	 3 5 10 10 7 15 10

(15.3) TABLE
Specifications of GAS-OIL

a/a	Type of Gas Oil / Properties	Automotive Gas Oil Limits	Heating Gas Oil Limits	ISO-F/DMA Marine (bunker) Gas Oil (c) Limits
1.	Density at 15 °C, kg/m³	820-845	to be reported	890 max
2.	Flash point °C min	55	55	60
3.	Sulfur, %wt, max	0.005	0.20	1.0
4.	Distillation			
	Recovered at 250 °C vol% max	65	-	
	Recovered at 350 °C, %vol, min	85	85	-
	Recovered at 380 °C, %vol, min	95	-	
5.	C.F.P.P. °C, min (a)			
	- *απο* 1/10 *εως* 31/3	-5	-5	-
	- *απο* 1/4 *εως* 30/9	+5	-	
6.	Pour Point °C max (a)			
	- *απο* 1/10 *εως* 31/3	-	-9	-6
	- *απο* 1/4 *εως* 30/9		0	0
7.	BS+Water %vol max	-	0.10	
8.	Colour (ASTM)	undyed	ASTM No3-No 5 (Red)	Black
9.	Water mg/kg, max	200	-	-
10.	Viscosity Kinematic at 40 °C cst	2.00-4.50	6	1.5 - 6.0
11.	Copper corrosion, ASTM No	1	3	-
12.	Suspended solids, mg/kg, max	24	-	-
13.	Carbon conradson residue, % wt, max	0.30 (b)	0.30 (b)	0.30 (b)
14.	Ash, % wt, max	0.01	0.02	0.01
15.	Oxidation of stability, g/m³, max	25	-	-
16.	Burning quality			
	Cetane number, min	51	-	40
	Cetane Index, min	46	40	-
17.	Solvent yellow 124 mg/kg	NIL	6	-
18.	Quinizarin ppm	-	-	3.0
19.	Polycyclic aromatic hydrocarbons, % m/m, max.	11		
20.	Lubricity corrected wear scar diameter (wsd 1.4) at 60 °C μm, max	460		

Παρατηρήσεις:

a. Τα Διυλιστήρια από 1/10 οφείλουν να παραδίδουν πετρέλαιο Κίνησης με χαρακτηριστικά χειμερινής περιόδου, παρότι επί δεκαπενθήμερο υπάρχει ανοχή στην λιανική πώληση πετρελαίου με θερινές προδιαγραφές.

b. Επί του 10% υπολείμματος απόσταξης.

c. *Για* πωλήσεις "automotive marine gas oil" ισχύουν *οι* προδιαγραφές *του* "automotive gas oil" *με* χρώμα "Μαύρο" *και* Κινιζαρίνη 3 mg/lt.