行政院所屬各機關因公出國人員出國報告書

（出國類別：進修　　）

中美合作計畫非游離輻射對環境與人體健康之影響

服務機關：行政院環境保護署

出國人職稱：聘用環境技術師

姓　　名：林怡君

出國地點：美　國

出國期間：九十年三月十七日至九十年三月卅日

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聯絡人／電話:

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出國人員:

林怡君　行政院環境保護署　空保處　環境技術師

出國類別: 進修

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關鍵詞: 非游離輻射,EPA,FCC,WHO,FDA

內容摘要: 近年來由於大哥大基地台之設置非常普遍，手機使用率大幅提高，有關非游離輻射對人體健康之影響議題亦逐漸受到重視。目前世界上大部分之國家皆是以聯合國世界衛生組織（WHO）所認定之ICNIRP,1998之標準爲其主要規範建議值；本署於九十年一月十二日公告之「我國非游離輻射環境建議值」亦是採用此標準作爲我國之標準。綜觀世界上目前有關此議題之研究結果,及此次出國研習所獲致之初步結論爲：國際上近一、二十年絕大部分之研究結果，並無明顯之證據顯示，長期或短期暴露於電磁輻射的環境下，會與某些特定生物效應（如腫瘤）有直接關係。未來我們亦將持續留意本議題之國際研究成果與發展趨勢，以隨時修正我國已公告之建議值，並與世界潮流接軌。

本文電子檔已上傳至出國報告資訊網

赴美國參加非游離輻射對環境及人體健康之影響計畫研習活動出國報告書

目錄…… 頁

壹、前言

本案係依據奉行政院核定「中美環保技術合作協定第四號執行辦法」執行之一「非（屬原子能）游離輻射對人體健康與環境之影響」計畫辦理。

本中美計畫案已於九十年二月十二日至二月十四日，由美國環保署（USEPA）楊仁泰博士，與美國環保署及聯邦通訊委員會（FCC）參與非游離輻射議題研究之資深專家，組成工作小組至台灣參訪，並與我國專家學者於九十年二月十三日舉行研討會；會中針對此議題進行廣泛之討論，並達到學術交流之目的。

由於我國已於九十年一月十二日完成非游離輻射環境建議值之公告，未來本署勢必將因應此議題民眾所產生之疑慮，且須配合國際上之研究結果及法令訂定趨勢修訂此建議值，故藉由本次派員至美國環保署、北卡州整合系統公司（ILS）、美國國家環境衛生科學研究所（NIEHS）、美國聯邦通訊委員會、美國食品藥物管理局（FDA）及摩托蘿拉公司（Motorola）等單位之總部或附屬非游離輻射實驗室研習活動，與美國非游離射研究領域資深科學家研習非游離輻射之研究現況，並收集目前國際上此議題之最新研究結果與管制方式，未來將可作為我國研修訂相關法規及推動非游離輻射相關業務時之參考。

貳、行程

日　　期	地　　點	工　作　內　容
九十年三月十七日（六）	台北	啟程
九十年三月十八日（日）	北卡羅來那州	抵達
九十年三月十九日（一）	北卡羅來那州	至北卡州美國環保署所屬實驗室研習與參訪。
九十年三月二十日（二）	北卡羅來那州	至美國國家環境衛生科學研究所（NIEHS）拜訪與至整合系統公司（ILS）參訪。
九十年三月二十一日（三）	北卡羅來那州－華盛頓 DC	行程
九十年三月二十二日（四）	華盛頓 DC	至美國環保署（USEPA）總署及輻射與室內空氣辦公室（ORI）參訪。
九十年三月二十三日（五）	華盛頓 DC	至美國聯邦通訊委員會（FCC）及食品藥物管理局（FDA）總部與相關實驗室研習及參訪。
九十年三月二十四日（六）	華盛頓 DC	收集資料
九十年三月二十五日（日）	華盛頓 DC－佛羅里達州	行程
九十年三月二十六日(一)	佛羅里達州	至美國摩托蘿拉總公司研習與參訪。
九十年三月二十七日(二)	佛羅里達州	至美國摩托蘿拉總公司研習與參訪。
九十年三月二十八日(三)	佛羅里達州	至美國摩托蘿拉實驗室研習與參訪。
九十年三月二十九日（四）	佛羅里達州	返程
九十年三月三十日（五）	台北	抵達

參、研習活動紀要

一、北卡羅來納州：

（一）至北卡州美國環保署所屬 RTP(Research Triangle Park)實驗室研習與參訪：

拜訪環保署科研處的布蘭克曼博士（Dr. Carl Blackman），並至其實驗室實地參訪與研討；經由與布蘭克曼博士討論後得知，在 60HZ 的電磁場強度下，利用細胞中的含鈣量作爲研究的基礎，他發現鈣的含量在一般電磁波情況下，沒有太大變化，但是在特殊的強度、特殊的地點，不同的周期的暴露率之下，可能對生物體會產生不同的影響。但目前這種現象還沒有一個確切的答案，也沒有合理的解釋。一些科學研究正在進行探討在這種暴露下可能發生負面的效應，可是在生物的實驗上還沒有一個固定的結果；所以根據他的實驗結果可分成下面幾點結論：第一點，有關電磁場對人體健康的影響程度依據目前結果尚不可斷然進行評斷；第二點，細胞對不同生物生化物理狀態的反應是不一致的，故必須以實際實驗的暴露情況才能作出確實的鑑定；第三點，動物實驗僅可以作為暴露實驗的參考。有關美國環保署 RTP 實驗室組織圖如附件一所示，實際實驗步驟如附件二、三所示。

（二）至北卡州整合系統公司（ILS）研習與參訪：

由環保署科研處的布蘭克曼博士陪同至整合系統公司拜訪虎克博士（Dr. Graham J. Hook）並至其實驗事實地參訪與了解實驗進行現況與

研究結果。虎克博士主要致力於進行無線電科技對細胞毒性之研究計畫（Wireless Technology Research WTR），依據虎克博士於討論時所提供之資料顯示，無線電作用於以沙門桿菌（Salmonella）及大腸桿菌（E.coli）等作為研究對象時，目前所得知之結論為｜無線電並不會造成此類物種之突變（mutation）或染色體之病變(chromosome aberration)及多倍化（polyploid），有關上述結論請參見附件四。

（三）至北卡州美國國家環境衛生科學研究所（NIEHS）拜訪：

藉由拜訪 NIEHS 組織內相關人員所提供之資料所得建議如下：相關研究建議大家應避免暴露於非游離輻射之環境下，並應擴大對民眾的宣傳，讓民眾了解科學領域裏已知和未知的情況，相關資訊可從 NIEHS 電力電磁場的問答題（Questions and Answers about EMF）所設立之網站www.niehs.nih.gov/emfrapid/home.htm上獲得相關資訊獲得，此外，亦可電洽由美國國家職業安全健康協會 NIOSH（National Institute for Occupational Safety and Health）所設立之熱線尋求解答。

二、華盛頓 DC：

（一）拜訪美國環保署（USEPA）總署及輻射與空氣辦公室（Office of Radiation and Indoor Air）：

於美國環保署總署會議室舉行會議，與會人員包括美國環保署總署楊仁泰博士與研發辦公室（Office of Research and Development）馬高希博士（Dr. Robert E.McGaughy）及輻射與空氣辦公室愛德華博士（Dr. Jon Edwards）。馬高希博士從事非游離輻射相關議題研究已逾三十年，於會議中建議台灣應該多參加國際性的電磁波（EMF）議題之相關會議，以獲得更多的知識，對於處理現有之爭議將可獲得更多的瞭解與背景知識，參加不同組織的會議，也可以增加對不同組織對電磁波標準不同態度之瞭解，以有利於未來台灣制定相關非游離輻射管制標準之參考依據；有關美國環保署研發辦公室之組織圖如附件五所示。

此外，輻射與空氣辦公室之愛德華博士針對非游離輻射於美國環保署之保護方式亦提出簡報及說明，目前非游離輻射之管制係屬空氣及輻射辦公室之輻射保護部門管轄，美國環保署空氣及輻射辦公室之組織圖如附件六所示；據愛德華博士簡報結果（簡報資料如附件七）顯示，目前美國環保署針對游離輻射有相關法規管制，然對於非游離輻射部分則上無任何法規或條文進行管制，相關非游離輻射設備之管轄分別是：大哥大基地台之設置及通訊器材之管轄單位為美國聯邦通訊委員會（FCC），醫院醫療設施及安全系統檢查等相關設備之管轄單位為美國食品藥物管理局（FDA）。美國環保署正研擬將非游離

輻射計畫納入游離輻射保護計畫中。

目前美國環保署對非游離輻射所施行之策略及政策僅限於進行科學研究、政策分析與資訊交流等項目，短期內美國環保署針對此議題將著重於教育大眾及提供充分之資訊，例如對大眾解釋，目前非游離輻射對人體健康之影響，迄今仍未有確切之研究成果，並構築相關網頁供民眾查詢，及持續進行相關研究以期得到更確定之結果，並將加強對社會大眾之教育，以減輕民眾對非游離輻射可能造成傷害之恐慌與疑慮，並教育民眾使其具有判斷媒體報導真偽之能力。

（二） 至美國聯邦通訊委員會（FCC）總部與相關實驗室研習及參訪：

於美國聯邦通訊委員會總部及其實驗室舉行會議及參訪；美國聯邦通訊委員會總部位於華盛頓 DC，其實驗室則位於馬里蘭州哥倫比亞區內，兩地相隔車程約一小時。

於美國聯邦通訊委員會總部會議時，據 FCC 克里夫蘭博士（Dr. Robert F.Cleveland）及馬丁波利博士（Dr. Edwin Mantiply）表示，目前美國所有相關非游離輻射如廣播電台等相關通訊設施之設置，皆必須符合 1996 年美國聯邦通訊委員會設定非軍用電臺的射輻（RF）標準，所有民用設施之設置必須向 FCC 申請執照，FCC 將於針對該設施實際量測，且於測值合乎射輻標準後核發使用執照。此標準是以 1986 國家輻射防護及量測委員會（NCRP）及 1992 年電機電子工程協會（IEEE）公佈之標準為基準。美國環保署亦同

意 FCC 的標準，可以保護人體健康，此項 FCC 公佈的規章可在聯邦法規第 47 集、第一部、1307 部分中查獲，美國聯邦通訊委員會亦將本冊聯邦法規贈送本署參考。

美國聯邦通訊委員會針對非游離輻射設置如下之網址供全球民眾查詢 www.fcc.gov/oet/rfsafety，民眾亦可透過熱線電話：1-202-418-2464 查詢；其餘於美國境內有關手機及無線電通訊器材之生產，亦必須符合 FCC 公佈的 SAR（比吸收率）的標準，FCC 公佈的 SAR 標準是根據 IEEE 及 NCRP 的放射標準制定，其規定手機之 SAR，係為每克身體組織的輻射量平均為 1.6W/kg。

於馬里蘭州哥倫比亞區美國聯邦通訊委員會實驗室會議時，由其實驗室主任尼可斯（Kenneth R. Nichols, Chief）進行簡報（簡報資料如附件八），目前該實驗室主要分為三大部門：一、設備認證；二、量測及校正；三、技術研發；該實驗時於 1941 年始於此處設立，從事技術性研究之目的主要係為建立新的管制政策；該實驗室除有相當完整之儀器設備外，針對射輻暴露量 SAR 值亦使用人體模型及模擬體液進行實驗，以檢測手機等相關電子產品對人體健康之實際影響。

（三）至美國聯邦食品藥物管理局（FDA）總部與相關實驗室研習及參訪：

美國聯邦食品藥物管理局（FDA）總部與相關實驗室皆位於華盛頓 DC，FDA 主要係針對食品及藥物與相關醫療設備進行管制，如微波爐電磁波之標準設定、醫院相關醫療設備如輪椅等與手機輻射之關係探討，與有關機場及相關大樓之電子安全檢查相關設施之檢測等與標準之訂定亦由該單位負責，據與 FDA 歐溫博士（Dr.Russell D. Owen）及班森博士（Dr. Howard I. Bassen）討論結果，目前於美國境內約有一億又八百萬民眾使用無線電話，每天更增加四萬六千名新使用者，有關手機輻射對人類健康之影響尚無定論，雖然某些使用轉殖基因之老鼠（transgenic mice）進行實驗所得之結果顯示，當其暴露於手機射輻照射下，產生淋巴病變之比例比未暴露於射輻照射者之兩倍，但仍無法直接判定其與人體健康有直接之關係。為解開此謎題，因此 FDA 正朝以下述方向努力以尋求解答：

1 於實驗室內進行針對 RF 刺激酵素活性實驗，並利用試管內（in vitro）及試管外活體試驗（in vitro）進行相互比較。

2 與通訊業者及網際網路相關組織研議開發一合作研發與通訊協定（Cooperative Research and Development Agreement CRADA）。

3 參與世界衛生組織（WHO）國際非游離輻射研究計畫。

4 參加相關準則研擬與開發團體，如 ICNIRP,IEEE SCC-28 及 SCC-34。

5 參與有關射輻研究之跨聯邦組織。

FDA 實驗室中主要進行電子安檢相關設備之檢測，於無響室中檢測非游離輻射相關設備所發出之電磁波對輪椅及心電圖等相關儀器之影響。

三、佛羅里達州：至美國摩托蘿拉總公司及實驗室研習與參訪

美國摩托蘿拉總公司及實驗室皆位於佛羅里達州勞德岱堡室，本次主要拜訪對象包括愛德華博士（Dr.Joe A Elder）、周重光博士（Dr.C.K.Chou）、史魏克博士（Dr.,Mays L.Swicord）及摩托蘿拉公司創辦人波然諾博士（Dr. Quirino Bolzono）；其實驗室中除針對手機之發射強度等進行檢測外，亦利用老鼠進行活體實驗及人體模型與模擬體液偵測人體對手機射頻 SAR 之影響，此部份實驗於 FCC 與 FDA 實驗室中皆可見相似之實驗儀器設備。

於拜訪上述相關博士所得非游離輻射電磁波對生物之效應，結論如下：

（一）在實驗室中，動物在高量 RF（射輻）輻射下之死亡率，係由熱效應造成。此乃因 RF 輻射造成生命不能承受的體溫極限。這在 5.7W/kg，1.5-4.0 小時的室溫下，由實驗毛猴、鼠及狗而獲得上述結論。

（二）對生物效應的影響，以瓦/公斤（W/kg）表示之比吸收率（SAR,Specific Absorpation Rate）比以能量強度豪瓦/平方公分（mW/cm2）者爲優。

（三）當周邊濕度較高時，SAR 的影響相對降低，對熱效應有影響的因素爲：濕度、氣流等環境因素，皆對生物反應有影響。

（四）在沒有熱效應時，RF 射輻對生命周期沒有顯著的負面效應。

（五）RF 射輻與癌症發生率沒有明顯關係。

上述博士亦提供如下之相關網址，可供國內人士查詢相關訊息時參考，

（一）www.mmfai.org

（二）www.fda.gov/cdrh/ocd/mobilephone.html/

（三）www.fda.gov/cdrh/phones

（四）www.radio.fer.hr

（五）ww.fgf.de

（六）www.icnirp.de

（七）www.nrpb.org.uk/backinfo.htm

（八）www.iegmp.org.uk

（九）www.who.int/peh-emf

肆、研習心得及建議：

近年來由於大哥大基地台之設置非常普遍，手機使用率大幅提高，有關非游離輻射對人體健康之影響議題亦逐漸受到重視，亦因對此議題之了解不清楚，故受報章雜誌之報導影響甚鉅，疑慮亦相當深遠。其實，要了解電磁輻射對人體之影響，必須就其頻率、強度等方面加以探討。其實一般所謂之輻射，可分為游離輻射及非游離輻射兩種，游離輻射指的是高於紫外線之輻射波，如X光、核子反應中所釋放之α、β、γ等射線，此游離輻射已被證實對身體有不良之影響，內含易造成細胞病變等致癌因素；而非游離輻射則指的是頻率再可見光以下之電磁波，電磁波對人體之效應可分為熱效應與非熱效應，熱效應會使組織昇溫，非熱效應則不會，但或許會與某些細胞生物效應有關。至於非游離輻射之民生應用，更是與民眾息息相關，成為不可或缺的一部份；例如在醫學界上所使用之雷射應用，在工業上所使用之工業切割、電腦視訊傳送、行動電話通訊及電力輸送設備等均為非游離輻射之使用範圍。近年來，有關非游離輻射的生物效應已逐漸被學術界廣加研究與探討，聯合國與許多先進國家亦紛紛著手訂定許多使用標準與相關管制法令與規範，以確保人們的健康。

目前世界上大部分之國家皆是以聯合國世界衛生組織（WHO）所認定之ICNIRP,1998之標準為其主要規範建議值；本署於九十年一月十二日公告之「我國非游離輻射環境建議值」亦是採用此標準作為我國之標準。

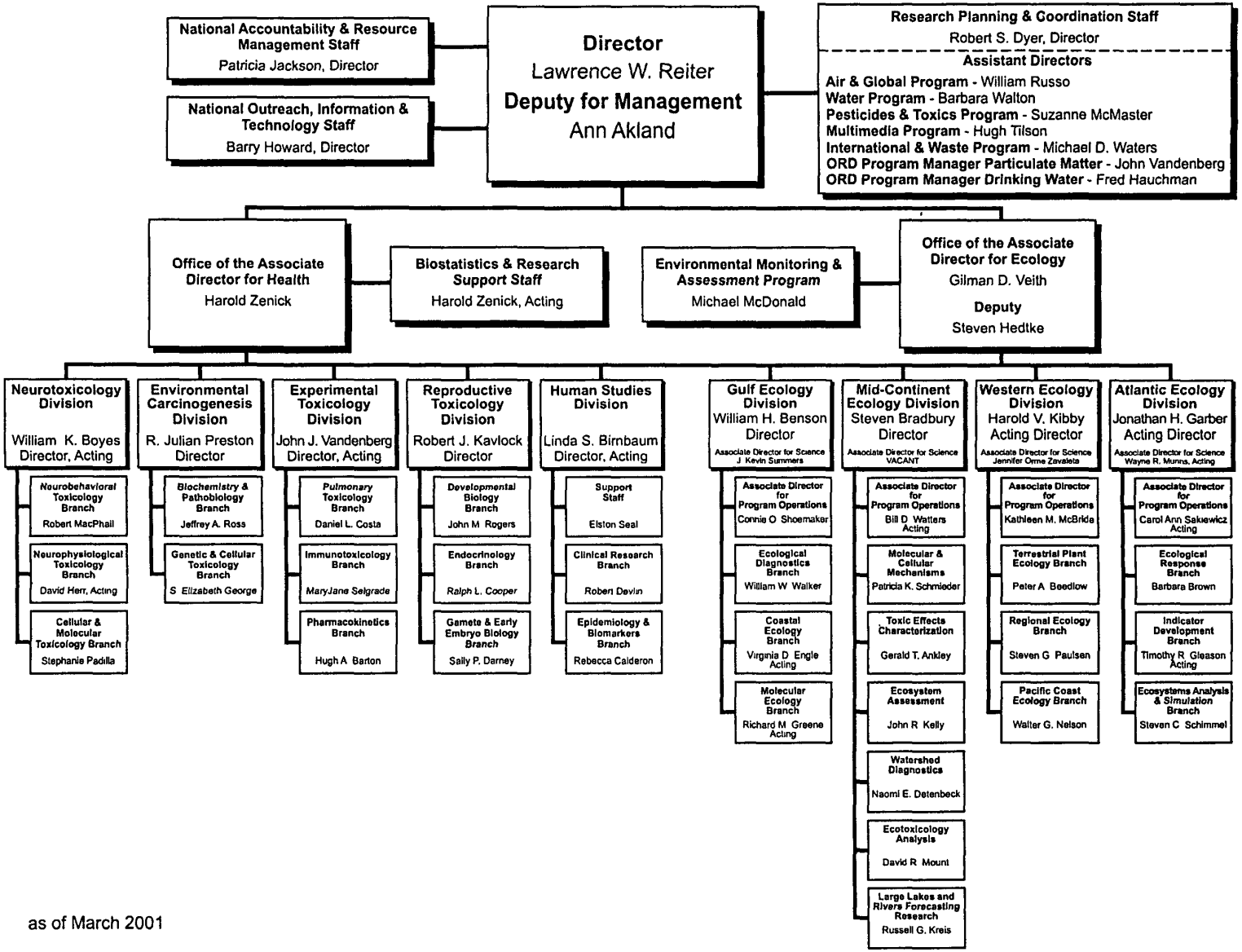
此次拜訪所得最大之感想為，非游離輻射對環境及人體健康之影響這個領域就像是一片大拼圖，每個研究人員或科學家就像是在完成一塊塊拼圖的成員，當此拼圖完成後，才能看出全幅成品之完整模樣，也才能解開這個謎題。

這個領域之研究正在世界上各個角落進行著，許許多多研究人員正兢兢業業的想將此謎題解開。綜觀國際上目前之研究成果，初步獲致之結論為：國際上近一、二十年絕大部分之研究結果，並無明顯之證據顯示，長期或短期暴露於電磁輻射的環境下，會與某些特定生物效應（如腫瘤）有直接關係。未來我們亦將持續留意本議題之國際研究成果與發展趨勢，以隨時修正我國已公告之建議值，並與世界潮流接軌。

附　　件

附件一　美國環保署 RTP 實驗室組織圖

National Health and Environmental Effects Research Laboratory (NHEERL-RTP, N.C.)



as of March 2001

Carl Blackmon's Lab

附件二　美國環保署 RTP 實驗室實驗步驟

OP NHEERL/ECD/BPB/CFB/96-03-0000

SCOPE/APPLICATION/LIMITATIONS

REQUIRED EQUIPMENT, SUPPLIES, PERSONNEL QUALIFICATIONS/TRAINING

PC12 cells

- Rat adrenal pheochromocytoma.
- American Type Culture Collection #CRL 1721.
- Respond reversibly to Nerve Growth Factor (NGF) by induction of neuronal phenotype.
- Cell line used for assay of NGF.
- Synthesize and store catecholamine neurotransmitters dopamine and norepinephrine but not epinephrine

Media & Supplements

- 85% RPMI Medium 1640 (Gibco#11875-051, formerly 320-1875AJ). [We do NOT add glutamine to this medium. We do not use the medium after the expiration date on the bottle]
- 5% Fetal Bovine Serum (Gibco #16000-028, formerly 240-6000AJ) [not heat inactivated].
- 10% heat inactivated Horse Serum (Gibco #16050-023, formerly 200-6050AJ) [we do the heat inactivation].
- 100 units/ml penicillin G sodium and 100 µg/ml streptomycin sodium (Gibco #15140-015, formerly 600-5140AG).
- as specified, Nerve Growth Factor (NGF) (2.5 S, Sigma #6009). NGF is prepared in Ca & Mg free PBS (but preparing in medium with serum will work too) in volumes and concentrations to be used in 1) priming, and 2) the experiment. We store our NGF solutions in the freezer.
- Type VII [Bornstein & Traub nomenclature] rat tail collagen [Classification Type I] (Sigma #C-8897), acid soluble.

CAUTION!

Do NOT put NGF into polypropylene tubes! NGF will stick to the polypropylene wall of such a tube and the concentration of NGF in solution will be indeterminate. Use only polystyrene tubes.

CAUTION!

Never filter sterilize any media component because medium components may stick to the filter and thus be removed or have their concentration in the solution be changed. If it is necessary to filter sterilize any medium component, carefully evaluate that process to ensure results subsequently obtained are consistent with published work.

[Note:

1. medium is at room temperature or slightly cooler when applied to cells.
2. The percent serum is constant in all experimental procedures, i.e., growth, prime, store, test.

ELF EXPOSURE SYSTEM

Our System

- Two 1000 turn coils of enameled wire on a plexiglass frame in a Helmholz configuration.
- 20 cm in diameter.
- Aligned coaxially 10 cm apart.
- Oriented to produce a vertical magnetic field.

- Two Configurations Used
  - #1. Single Coil Configuration for alternating magnetic field
    - Only bottom coil energized.
    - A maximum of 9 dishes were stacked on top of each other in the center of the coils.
    - Produced a diminishing intensity from bottom to top.
  - #2. Double coil configuration for alternating magnetic field
    - Coils connected and both energized
    - Platform placed in coils level with top of bottom coil.
    - Magnetic field produced in same direction
    - Relatively uniform flux density in a region in the middle of the exposed dishes.
- For exposure to gradient of alternating-current magnetic field intensity, use configuration #1. Energize both coils with direct current (DC) and only the lower coil with alternating current (AC) at the frequency and intensities desired, based on results desired using equations provided in Blanchard and Blackman (Bioelectromagnetics 15:217-238, 1994) and Blackman et al. (Bioelectromagnetics 15:239-260, 1994; Bioelectromagnetics 19:204-209, 1998 and citations therein). Exposed dishes should be placed in the uniform field region of configuration #1 as determined by standard techniques (see Misakian et al., Bioelectromagnetics Supplement 2:1-73, 1993). Control dishes should be placed in a separate incubator without energized coils, or in the same incubator inside a co-netic shield.
- For exposure to uniform intensity magnetic fields, use configuration #2. Energize the coils with both direct current (DC) and alternating current (AC) at the frequency and intensities desired, based on results desired using equations provided in Blanchard and Blackman (Bioelectromagnetics 15:217-238, 1994) and Blackman et al. (Bioelectromagnetics 15:239-260, 1994; Bioelectromagnetics 19:204-209, 1998 and citations therein). Exposed dishes should be placed in the uniform field region of configuration #2 as determined by standard techniques (see Misakian et al., Bioelectromagnetics Supplement 2:1-73, 1993). Control dishes should be placed in a separate incubator without energized coils, or in the same incubator inside a co-netic shield.

Other magnetic field exposure systems

Kirschvink (Bioelectromagnetics 13:401-411, 1992) has described other coil configurations that will lead to similar exposure conditions as those developed in our laboratory.

CAUTIONARY NOTES AND SPECIAL CONSIDERATIONS:

Do NOT put NGF into polypropylene tubes! NGF will stick to the polypropylene wall of such a tube and the concentration of NGF in solution will be indeterminate. Use only polystyrene tubes.

Never filter sterilize any media component because medium components may stick to the filter and thus be removed or have their concentration in the solution be changed. If it is necessary to filter sterilize any medium component, carefully evaluate that process to ensure results subsequently obtained are consistent with published work.

Do not place the dishes in the immediate proximity of any circulating fan motor that may be in your incubator because the motor produces magnetic fields that may influence your cell response.

PROCEDURE:

Growth

- Cells grown in plastic 75cm^2 cell culture flasks (T75) (Costar #3075). These flasks are not collagen coated.
- 5% CO_2 at 37^{o}C.
- Subcultured when 80% confluent.

Subculture

- Prepare three T75 flasks (without collagen coating) by labeling with cell line, passage number and date.
- Add 10 ml sterile media to each of the T75 flasks (without collagen coating).
- From Stock Culture of PC12 cells growing in a T75 flask (80% confluent), pour off media.
- Rinse with 2 ml 0.25% Trypsin, decant immediately, leaving a thin film of solution on the cells) (Gibco #15050-016, formerly 610-5050AG) [trypsin only in HBSS, e.g., no EDTA].
- Incubate at 37^{o}C for 5 minutes.
- Bang flask against vertical palm of hand to dislodge cells [do not use chemicals to encourage cell removal].
- Add 15 ml media to resuspend the cells.
- Triturate vigorously 25 times to break up clumps.
- Put 5 ml resuspended cells in each of the prepared T flasks.
- Leave caps loose and swirl to get an even distribution of cells.
- Place in 5% CO_2 incubator at 37^{o}C.
- Check daily for growth.
- Subculture again when 80% confluent.
- Usually subculture Monday and Friday.
- Cells subcultured Friday are usually used Monday, Tuesday, or Wednesday for experiments not requiring primed cells.
- Cells subcultured Monday are usually used Wednesday or Thursday for experiments not requiring primed cells.
- For experiments requiring primed cells, see below.

Preparing Primed Cells

- Primed cells were prepared in three 6-well plates (Costar#3406).
- The 35-mm diameter wells were coated with 0.25 ml collagen, allowed to dry and rinsed with 1 ml of media just prior to use. **See "1. Prepare collagen coated dishes ..." on page 4 for details.**
- The PC12 cells were removed from one 80% confluent T75 flask (without collagen coating); see subculturing information (above), but without steps 4 & 5, i.e., the Trypsin & incubation steps. However, some cells may require a trypsin treatment; there should be no problem using this step as along as the cells subsequently respond in a manner as shown in Rukenstein and Greene (Brain Res. 263:177-180, 1983) to a concentration series of NGF (see also Greene, Brain Research 133:350-353, 1977).
- The cells were diluted to 2x10^4 cells per ml in media (count with hemacytometer).
- The first well on each 6-well plate was labeled "**C**" and received plain cells only, with no NGF, thus making it a growth control.
- The other 5 wells on each plate received 2 ml cells to which NGF had been added to a concentration of 50 ng/ml.
- The day that the priming was started was designated day 1.
- The media on the plates was changed on Day 3, 5, and 7.
  - On the designated day [3], 1 ml of media was removed from each well of every plate. By starting with the well labeled "C", the same pipet can be used for the remaining wells of each plate without getting NGF into the growth control wells, "C".
  - One ml of fresh media was added to each of the "C" wells.

- NGF was added to 15 ml media to give 100 ng/ml NGF. One ml of the NGF-supplemented media was added to each of the remaining wells, because NGF activity is drastically reduced after two days on cultures maintained at 37 °C. This process will lead to a final NGF concentration of 50 ng/ml.
- This replenishment schedule was repeated on days 5 and 7.

• On day 8, all the media was removed from all the wells (The "**C**" wells were not processed further, but were inspected for normal cell morphology as a quality control).
• Each NGF well received 2 ml media, was gently swirled and media removed with a pipet to wash off excess NGF.
• This washing procedure was repeated twice more for a total of 3 washings.
• After the final washing 2 ml of medium was added to each well and the medium gently pipeted up and down to remove all the cells. [do not use chemicals to encourage cell removal]
• The media with cells were placed in two 15 ml centrifuge tubes.
• The cells were centrifuged at 500 rpm (30xg) for 10 minutes to pellet the cells.
• The media was poured off and the cells were resuspended in 10 ml media and counted by Coulter Counter or hemacytometer.
• The cell suspension was diluted to give 8×10^5 cells per ml in medium which is adjusted to contain a final amount of Dimethyl Sulfoxide (DMSO) at 10%. Note, this medium contains serum; we did not follow any procedure where serum is excluded from cell samples before freezing.
• One ml samples were frozen slowly in freezer vials placed in a double-walled styrofoam box in a -80^{o}C freezer.
• Use as described in "**Using Primed PC12 in Experiments**".
• Perform an assay of cell response to NGF for each set of primed cells to ensure the cell responses are as described in Rukenstein and Greene (Brain Research 263:177-180, 1983). Should the cells not respond as described by this reference, they should be destroyed in order to maintain quality control, see below.

CONFIRMATION OF EXPTL TECHNIQUE & QUALITY CONTROL - NGF DOSE RESPONSE:

See Greene LA and Tischler AS (1976) Proc. Natl. Acad. Sci. (USA) 73:2424-2428, Greene LA (1977) Brain Research 133:350-353,
Rukenstein A and Greene LA (1983) Brain Research 263:177-180.

1. Prepare collagen coated dishes in a bioguard hood before beginning experiment

• The standard petri dish used is a 60 mm (Costar #3060).
• The collagen used is Type VII [Bornstein & Traub nomenclature] rat tail collagen [Classification Type I] (Sigma #C-8897), acid soluble.
Prepare using sterile technique.
Dissolve 5 mg collagen in 125 ml of 0.1 M acetic acid
Mix on stirrer until dissolved, approximately 40 minutes.
Divide into bottles (we normally put 50 ml into a clear, 125-ml bottle) and store in refrigerator. [do not filter sterilize]
• Put 0.45 ml collagen in each 60 mm dish needed for an experiment. See table below for other dish types.
• Spread with a sterile glass rod to cover the bottom surface of the dish.
• With the tops ajar, let air dry in a bioguard hood until dry, about an hour and a half.
• Just before use rinse each dish with 1.5 ml media which adjusts the pH. [we have never tried to store collagen coated dishes in refrigerator or freezer for later use]

Amount of Collagen needed to Coat Different Dishes

Dish Size	Manufacturer	Amount of Collagen	Rinse Volume
35 mm	Costar #3035	0.25 ml	1 ml
6 well plate	Costar #3406	0.25 ml	1 ml
60 mm	Costar #3060	0.45 ml	1.5 ml
100 mm	Corning #25020	1 ml	5 ml
organ culture	Falcon #3037	inside 0.1 ml outside 0.3 ml	inside 0.5 ml outside 1.5 ml

2. Using Primed PC12 in experiments

- Use one vial of frozen-primed PC12 cells for every 8 dishes (40 ml).
- Thaw vial in 37°C water and rinse with 65% ethanol before opening.
- Resuspend the cells in the vial in 5 ml media in a 15 ml centrifuge tube (Costar #3215). Rinse vial with 1 ml of the 5 ml. Triturate ~2x.
- Centrifuge cells at 500 rpm (30xg) for 10 minutes to pellet all the cells.
- Pour off supernatant, tap tube several times to dislodge pellet, resuspend in 6 ml media, triturate 5-10x, spin again.
- Repeat above once more for a total of 3 rinses.
- Resuspend cells in 3 ml, triturate 20x to break up clumps, added to medium to give total of 41 ml at $2x10^4$ cells per ml for 60 mm dishes. The aim is to get ~20 cells per microscope field at 200x.
- Put 5 ml of cell prep without NGF in each rinsed collagen-coated control dish.
- To the rest of the cell suspension add NGF to give a range of concentrations such as 1, 2.5, 5, 7.5, 10, 15, 25 and 50 ng per ml NGF, mix and add to rinsed, collagen-coated dishes. A response similar to described by Rukenstein and Greene (Brain Research 263:177-180, 1983) is acceptable. For example, an initial sharp rise in % cells scoring positive for neurite outgrowth (%NO) should reach a peak by 5-7.5 ng/ml, but the response may continue higher at higher concentrations of NGF. The % NO should be between 60 and 75 % or more at NGF concentrations of 15 to 50 mg/ml.
- Select the concentration of NGF that causes 60-75% of the maximum cell response to NGF determined in the previous step, above. For our cells, that concentration is 5 ng/ml. For your cells you may need to use a different concentration of NGF.

CAUTION!
Do not place the dishes in the immediate proximity of any circulating fan motor that may be in your incubator because the motor produces magnetic fields that may influence the response of your cells.

Volume of Cells Used in Different Dishes

Dish size	Manufacturer	Volume of Media
35 mm	Costar #3035	2 ml
6 well plate	Costar #3406	2 ml
60 mm	Costar #3060	5 ml
100 mm	Corning #25020	25 ml
organ culture	Falcon #3037	inside 1 ml outside 3 ml

3. Incubation of Cells

- Place dishes in a CO_2 incubator at 37°C.
- All samples in an experiment should be placed in the incubator within 5 minutes after plating.
- Incubate for 23 hours.

- At the end of that time the cells with and without neurites were counted or the cells were fixed for counting later (media was removed from each dish and 2 ml of 1.25% glutaraldehyde was applied and they were stored in a closed plastic bag until they could be counted).

4. Assaying Neurite Outgrowth in PC12 Cells

- Cells were assayed using an inverted stage microscope with a phase contrast lens at a magnification of 200x.
- Entire, non-overlapping microscopic fields of cells were counted so the total number of cells counted varied. Overlapping fields (ergo double counting of the same cells) should be avoided.
- At least 100 cells were counted per dish.
- Cells were scored as having a neurite (positive) or not (negative).
- A protrusion from a cell was considered a neurite if it were at least as long as the diameter of the cell or if it were branched or if it had a growth cone (growth cones appear as small ball shapes at the end of neurites which appear clear in the center under phase contrast; less than 5% of the positive scores were due to growth cones). This response is what we have experienced as typical for our experiments. If your results deviate from this by 20% or more, then cells from other sources should be tested.
- PC12s tend to clump; clumps were counted as one cell.
- If a clump had at least one neurite protrusion which was considered positive, then the entire clump was scored as one positive.
- If a clump had no neurite protrusions which were considered positive, then the clump was scored negative.
- The number of cells with neurites was divided by the total number of cells counted per dish to give the % cells with neurites.

EXPERIMENTAL EXPOSURE TO MAGNETIC FIELDS OR TO MAGNETIC FIELDS AND A CHEMICAL:

1. Prepare collagen coated dishes in a bioguard hood before beginning experiment

- The standard petri dish used is a 60 mm (Costar #3060).
- The collagen used is Type VII [Bornstein & Traub nomenclature] rat tail collagen [Classification Type I] (Sigma #C-8897), acid soluble.
  Prepare using sterile technique.
  Dissolve 5 mg collagen in 125 ml of 0.1 M acetic acid
  Mix on stirrer until dissolved, approximately 40 minutes.
  Divide into 50-65 ml amounts in clear, 125-ml bottles and store in refrigerator.
- Put 0.45 ml collagen in each 60 mm dish needed for an experiment. See table below for other dish types.
- Spread with a sterile glass rod to cover the bottom surface of the dish.
- With the tops ajar, let air dry in a bioguard hood until dry, about an hour and a half.
- Just before use rinse each dish with 1.5 ml media which adjusts the pH.

Amount of Collagen needed to Coat Different Dishes

Dish Size	Manufacturer	Amount of Collagen	Rinse Volume
35 mm	Costar #3035	0.25 ml	1 ml
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organ culture	Falcon #3037	inside 0.1 ml outside 0.3 ml	inside 0.5 ml outside 1.5 ml

2. Using Primed PC12 in experiments

- Use one vial of frozen primed PC12 cells for every 8 dishes (40 ml).
- Thaw vial in 37°C water and rinse with 65% ethanol before opening.
- Resuspend the cells in the vial in 5 ml media in a 15 ml centrifuge tube (Costar #3215). Can rinse vial with 1 ml of the 5 ml. Triturate ~2x.
- Centrifuge cells at 500 rpm (30xg) for 10 minutes to pellet all the cells.
- Pour off supernatant, tap tube several times to dislodge pellet, resuspend in 6 ml media, triturate 5-10x, spin again.
- Repeat above once more for a total of 3 rinses.
- Resuspend cells in 3 ml, triturate 20x to break up clumps, added to medium to give total of 41 ml at $2x10^4$ cells per ml for 60 mm dishes. The aim is to get ~20 cells per microscope field at 200x.
- Put 5 ml of cell prep without NGF in each rinsed collagen-coated control dish.
- To the rest of the cell suspension add NGF to give 5 ng per ml NGF, or the amount of NGF to produce 60-75% of the maximal cell response (see CONFIRMATION OF EXPTL TECHNIQUE & QUALITY CONTROL - NGF DOSE RESPONSE, #2, above), mix and add to rinsed, collagen-coated NGF control and exposure dishes.
- Add desired amount of a test chemical into appropriate control and magnetic-field exposed dishes as per experimental design. If more than one vial of frozen primed PC12 cells is needed, add contents of these vials together and mix before dispensing.
- Place dishes in treatment/control positions in exposure system.

Volume of Cells Used in Different Dishes

Dish size	Manufacturer	Volume of Media
35 mm	Costar #3035	2 ml
6 well plate	Costar #3406	2 ml
60 mm	Costar #3060	5 ml
100 mm	Corning #25020	25 ml
organ culture	Falcon #3037	inside 1 ml outside 3 ml

3. Exposure of Dishes to Magnetic Fields

- The ELF exposure system and shielded controls were all placed in a CO_2 incubator at 37°C.
- Controls were placed in a co-netic metal (an alloy with high magnetic field susceptibility properties) container in the same incubator, or a different incubator. The co-netic metal absorbs the ambient magnetic fields, both from the external environment and from the incubator and exposure system, and thus shields the control cells from magnetic field exposure.
- All samples in an experiment were placed in the exposure housing within 5 minutes after plating and the fields were then turned on.
- Exposures were for 23 hours.
- At the end of that time the cells with and without neurites were counted. The cells can be fixed for counting later (media was removed from each dish and 2 ml of 1.25% glutaraldehyde was applied and they were stored in a closed plastic bag at room temperature until they could be counted). Counting should be done within several days and care should be taken to ensure cells and neurites have not become detached. Observation of the cells through a microscope that shows cells floating and only fixed to the dish at the neurite ends when the dishes are slowly moved, have been left too long and should be discarded without counting.
- For exposure to gradient of alternating-current magnetic field intensity, use configuration #1. Energize both coils with direct current (DC) and only the lower coil with alternating current (AC) at the frequency and intensities desired, based on results desired using equations provided in Blanchard and Blackman (Bioelectromagnetics 15:217-238, 1994) and Blackman et al. (Bioelectromagnetics 15:239-260, 1994; Bioelectromagnetics

19:204-209, 1998 and citations therein). Exposed dishes should be placed in the uniform field region of configuration #1 as determined by standard techniques (see Misakian et al., Bioelectromagnetics Supplement 2:1-73, 1993). Control dishes should be placed in a separate incubator without energized coils, or in the same incubator inside a co-netic shield.

- For exposure to uniform intensity magnetic fields, use configuration #2. Energize the coils with both direct current (DC) and alternating current (AC) at the frequency and intensities desired, based on results desired using equations provided in Blanchard and Blackman (Bioelectromagnetics 15:217-238, 1994) and Blackman et al. (Bioelectromagnetics 15:239-260, 1994; Bioelectromagnetics 19:204-209, 1998 and citations therein). Exposed dishes should be placed in the uniform field region of configuration #2 as determined by standard techniques (see Misakian et al., Bioelectromagnetics Supplement 2:1-73, 1993). Control dishes should be placed in a separate incubator without energized coils, or in the same incubator inside a co-netic shield.

4. Assaying Neurite Outgrowth in PC12 Cells

- Cells were assayed using an inverted stage microscope with a phase contrast lens at a magnification of 200x.
- Entire microscopic fields of cells were counted so the total number of cells counted varied.
- At least 100 cells were counted per dish.
- Cells were scored as having a neurite (positive) or not (negative).
- A protrusion from a cell was considered a neurite if it were at least as long as the diameter of the cell or if it were branched or if it had a growth cone (growth cones appear as small ball shapes at the end of neurites which appear clear in the center under phase contrast; less than 5% of the positive scores were due to growth cones).
- PC12s tend to clump; clumps were counted as one cell.
- If a clump had at least one neurite protrusion which was considered positive, then the entire clump was scored as one positive.
- If a clump had no neurite protrusions which were considered positive, then the clump was scored negative.
- The number of cells with neurites was divided by the total number of cells counted per dish to give the % cells with neurites.

Notes:

-Comments, suggestions, or questions should be addressed to:

Carl Blackman, EPA (MD-68), Research Triangle Park, NC 27711 USA
phone: 919-541-2543, fax: 919-541-1477, (alternative -0694)
email, internet: Blackman.Carl@epa.gov

附件三　美國環保署 RTP 實驗室實驗步驟

Intramural Research Protocol Form

Nerve Growth Factor Action - cell culture
NHEERL-H/ECD/BPB/CFB/2000-01-0000

Project/Task Organization, Problem Definition/Background

US EPA is interested in understanding the basic mechanisms by which chemically induced events can lead to tumor formation, so that risk assessment can be conducted in a more scientifically informed manner. Tumor formation is generally believed to occur as a consequence of two events, initiation and promotion. Initiation is the alteration of DNA information. Promotion is the series of events that occur following initiation, and it involves changes in information processing mechanisms that lead to uncontrolled growth. Our research, using biochemical and biophysical techniques, investigates the influence of chemicals on information transfer at the plasma membrane level. We are examining the influence of growth factors on cell growth control and differentiation to explore the means by which chemical agents can interfere with membrane signaling processes. In this effort, we use magnetic fields as a biophysical tool to aid in the elucidation of the mechanistic bases for chemically induced changes in signaling processes.

We have developed and are now refining the use of magnetic fields to perturb on-going membrane-based phenomenon. We are using PC-12 cells, a pheochromocytoma cell from the rat adrenal medula, and its neurite outgrowth in response to stimulation by nerve growth factor for study in this project. Neurite outgrowth, a differentiation response demonstrating growth control, has been used in our laboratory to study the effects of alkylated tins, which are model toxicological agents that are also of interest to EPA because of their presence in estuaries. The neurite outgrowth assay is simple, requiring very little sophisticated equipment, and it is well established, having been used in over 200 laboratories around the world since its development by Greene and Tischler [1976). The assay is described in the attached operating protocol NHEERL-H/ECD/BPB/CFB/96-03-0000.

Our initial finding in a study of magnetic field strength dependence, was that magnetic fields as low as 22 mGrms, but not the corresponding induced electric field/current, was responsible for stimulating neurite outgrowth in a sub-clone of PC-12 cells, called PC-12D. Magnetic fields stimulated neurite outgrowth in the absence of NGF [Blackman et al., 1993a], and pilot data demonstrated that magnetic fields would augment the stimulatory capability of sub-optimal concentrations of NGF to cause these cells to produce neurites. We also tested the parental line, PC-12, to see if they would be similarly sensitive to magnetic field exposure, and discovered that magnetic fields would only influence neurite outgrowth in these cells, in a field-strength dependent study, when the cells were primed and also stimulated with NGF. Again, only the magnetic field, and not the induced electric field, was responsible for alteration in neurite outgrowth. The parental PC-12 cell response was the reverse of the sub-clone, namely, fields caused the inhibition of NGF-stimulated neurite outgrowth [Blackman, et al., 1993b]. These results gave us confidence that magnetic fields can reliably alter a membrane-based growth factor process in two different cells, and thus may be used as an adjunct to more traditional assays to determine underlying mechanism(s) of action.

Further work was needed to characterize all of the magnetic field conditions responsible for these effects so that we could fully develop magnetic fields as a tool to study biological processes, including alterations caused by chemical agents. This approach also has the benefit of making any independent replication of results much more likely, thereby eliminating some the difficulties of avoidable controversy. For example, we decided to examine the potential influence of different closely related frequencies on our neurite inhibition effect in PC-12 cells, and observed a frequency-dependent change in the field-strength-dependent response of the cells [Blackman et al., 1995]. This result was similar to previous results with another biological system, and this motivated us to consider other physical agents as potential co-factors.

We designed an experiment to test the possibility that the static (DC) magnetic field generated by the earth and modified by technology-based apparatus could influence this frequency dependency. Our results indicated that the parallel components of the AC and DC magnetic fields were interacting to alter the cell responses to exposure. After establishing a collaborative relationship with a physical scientist at the Bechtel Corporation, we were able to correct, revise and expand an interaction model [Blanchard and Blackman, 1994a], which we termed the Ion Parametric Resonance (IPR) model. We then experimentally confirmed the major predictions of the IPR model with the PC-12 cell assay system [Blackman et al., 1994]. These results were combined in a book chapter written for a more general audience [Blanchard and Blackman, 1994b]. The predictions of the IPR model, and the tests with the PC-12 cell assay system, gave remarkable correspondence between prediction and experimental results, and have led to additional studies. The predictions of the IPR model were tested further by extending the field-strength range, and the results were essentially as predicted by the model [Blackman et al., 1995].

Analysis of the initial data set using the IPR model predictions had an unexpected outcome. A post-hoc evaluation indicated that hydrogen, an ion never mentioned by any previous publication as potentially involved in direct magnetic field interactions, may have played a role as a trigger ion, perhaps to destabilize molecular structures so that they would be more sensitive to field interactions with other associated ions. We tested this hypothesis by deliberately tuning only for the hydrogen ion, and obtained results that were generally consistent with IPR model predictions, except for a response change in the region of optimum predicted response [Trillo et al., 1996]. The identification of magnetic field exposure conditions that affect hydrogen in biological systems, without changing global pH, has opened a new avenue to study chemical interactions on cells. Additional study showed the bandwidth for this process was consistent with a resonance-type interaction [Blanchard et al., 1994].

The IPR model was also used to evaluate published data of responses by a exposed animal system, which resulted in recommendations for definitive ranges of parametric manipulations for future studies [Blanchard et al., 1995].

Project/Task Description

The main objectives of this work are to examine signal transduction processes associated with cancer promotion. The signal transduction processes associated with growth factor stimulation are studied to determine whether and by what means chemical agents alter that information flow influencing cell differentiation and proliferation. Further, studies will determine if a physical agent, magnetic fields, can be used to reveal more information concerning these chemical perturbations, particularly tumor promoting activities of EPA regulated agents. Insight into the mechanistic basis and relative potencies for chemically induced changes in growth factor action is sought. Research employs molecular and biochemical techniques. Because magnetic fields pass through biological materials virtually unchanged, it is realistic for other investigators to consider using exposure conditions established by this protocol for producing biological responses using in vitro samples, to test responses on in vivo exposed specimens, e.g., intact animals.

The research being performed has two distinct focuses: to examine the magnetic field and chemical conditions that are responsible for the changes demonstrated by the PC-12 cell assay, and to identify the region in the cell and/or the substrate that responds to the magnetic field exposure.

The IPR model has identified ions that are potentially responsive to magnetic fields. We have treated the PC-12 cell system under magnetic field exposure conditions that the IPR model predicts correspond to some of these ions. Additional study will be left to other scientists to obtain an inventory of all ions that are active so that other more specific biological test systems, i.e., subcellular molecular preparations, can be selected for testing to allow a more detailed refinement of the IPR model.

Another approach is to dissect the biological basis for the response being observed in the PC-12 cell assay. We have begun to use different stimulatory agents that are known to act at different sites within the cell to induce neurite outgrowth and test their susceptibility to magnetic field exposure [Blackman et al., 1993c]. We have also examined the influence of melatonin, a natural hormone which has oncostatic properties, on biochemical transduction events that may be changed in cells exposed to magnetic fields. Several studies have demonstrated that this approach is feasible and potentially useful [Ubeda et al., 1996; Blackman et al., 1997].

Test Substance

Chemicals selected for this study will include hormonally active agents, including melatonin, that have known or putative actions to influence cancer promotion processes. Depending upon the value of research results obtained, halide disinfection by-products, particularly DCA, TCA and other haloacid analogues and metabolites, could become a focus of this research to determine the mechanism(s) by which transmembrane signal transduction is altered. A model tumor promoter, the phorbol ester, TPA, will be used a comparative cancer promoting agent.

Quality Objectives and Criteria for Measurement Data

See OP/protocol NHEERL-H/ECD/BPB/CFB/96-03-0000

Special Training /Certification

No Special training needed.

Documentation and Records

Records should be created for magnetic exposure conditions, biological specimen description and handling conditions, chemicals used in the study, biological response(s) to positive and negative controls and to the specific magnetic field conditions. Data should be stored as paper copy or magnetic media copy. Our storage is paper copy as per Agency Records Schedule RNDL 503.

Experimental Design

The exposure system should be of a design to produce both uniform and gradient magnetic fields, of varying intensity and frequency, including static fields, in the volume where the biological samples will be exposed. The exposure values should not vary uncontrollably by more than 2% over the volume of exposure. In the case of neurite outgrowth from PC-12 cells in culture, a pair of Helmholtz-aided coils inside a CO_2 incubator were sufficient. The number of biological samples to be exposed for any given magnetic field condition will depend on the magnitude and variability of the biological response measured. Positive control agents of comparable effect should be used to establish sensitivity of the biological endpoint being monitored, and to develop an exposure protocol to give an estimate of the sample numbers needed. After consultation with a professional statistician, pilot data should be obtained for magnetic field effects and analyzed for sources of error. Protocols should be revised to optimize statistical power while minimizing research efforts and use of resources. In the PC-12 cell assay, it was sufficient to test each exposure condition with three different frozen samples of cells.

Sampling Methods, Sample Handling and Custody

Cell cultures should be frozen and stored at liquid nitrogen temperatures to ensure integrity of biological specimen. Animals should be obtained from established suppliers who maintain the genetic status of the animals.

Analytical Methods

See attached OP NHEERL-H/ECD/BPB/CFB/96-03-0000 for PC-12 Neurite Outgrowth assay.

Quality Control

See attached OP NHEERL-H/ECD/BPB/CFB/96-03-0000 for PC-12 Neurite Outgrowth assay. The NGF potency and quality is tested for every batch of primed cells that are made to establish the concentration needed to provide the expected response, i.e., 50% scoring positive for neurite outgrowth in control conditions. Cell cultures should be frozen and stored at liquid nitrogen temperatures to ensure integrity of biological specimen. Animals should be obtained from established suppliers who maintain the genetic status of the animals.

Instrument/Equipment Testing, Inspection, and Maintenance

Use historical values for setting each time experiments are run.

Instrument/Equipment Calibration and Frequency

Check frequency and intensity of electric currents using at least three Fluke multimeters two times per year using at least two different frequency generators. Historical settings are used each time experiment run to ensure conditions.

Check magnetic field intensity in Helmholtz coil calibration apparatus at least once every three years. Use multiple fluxgate probes in same magnetic field yearly to assure constancy of measurements - fluxgate probes are very stable. Historical setting of current are used to ensure conditions each time experiment run.

Inspection/Acceptance of Supplies and Consumables

Supplies are purchased from reliable vendors who provide certification that company quality procedures have been followed. Furthermore, control experiments are performed to ensure that the assay system is responding according to historical records and published literature.

Non-direct Measurements

Data from others should include results from triplicate cell platings, control results using a series of concentrations of NGF from 0.5 to 30 ng/ml, exposure results when NGF concentrations only stimulate 50-65% of maximal possible neurite outgrowth, dosimetry for static and AC field intensities, alignments, and AC frequency. Descriptions should also include the source and kind of medium and serum used, the cell concentration plated, and the method of neurite outgrowth assay used. See OP for more details.

Data Management; Data Review, Verification, and Validation

Calculations/measurements are described in the OP. Analysis depends on the focus of the experiments, and should be established in collaboration with a professional statistician. For on-resonance/off-resonance experiments, a one-way analysis of variance should be sufficient. For more sophisticated analyses, see peer-reviewed publications, e.g., Blackman et al. 1994.

Assessments and Response Actions

If collaborating with scientists outside the Agency in RTP, it may be necessary for visits to labs to see facilities and discussion differences and their possible impact, and ways to test them.

Reports to Management

Unnecessary.

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附件四　北卡州整合系統公司（ILS）虎克博士研究結論

→ Geogre Carlo

WTR Toxicology Program: Summary of Results (GJH March 20, 2001)

ISL Graham Hook.

Technology	Assay (Endpoint)	Exp.	Result	Conclusion
Analog	Salmonella/E. coli (Mutation)	SRI 1	Negative	Not mutagenic
		SRI 2	Equivocal TA1537	
		ILS 1	Negative	Not mutagenic
		ILS 2	Equivocal TA1535, TA98	
	MOLY (Mutation)	SRI 1	Negative	Negative response
		SRI 2	Negative	
		ILS 1	Negative	Not mutagenic
		ILS 2	Negative	
	HULY (Chromsome aberrations)	SRI 1	Negative	Negative
		SRI 2	Negative	
		ILS 1	Negative	No induction of chromosome aberrations or polyploidy
		ILS 2	Negative	

31

4 types of technology

WTR Toxicology Program: Summary of Results (GJH March 20, 2001)

Technology	Assay (Endpoint)	Exp.	Result	Conclusion
TDMA	Salmonella/E. coli (Mutation)	ILS 1	Negative	Not mutagenic
		ILS 2	Negative	
	MOLY (Mutation)	ILS 1	Negative	Not mutagenic
		ILS 2	Negative	
	HULY (Chromsome aberrations)	ILS 1	Negative	No induction of chromosome aberrations or polyploidy
		ILS 2	Negative	
CDMA	Salmonella/E. coli (Mutation)	ILS 1	Equivocal TA1535	Not mutagenic
		ILS 2	Negative	
	MOLY (Mutation)	ILS 1	Equivocal	Not mutagenic
		ILS 2	Negative	
	HULY (Chromsome aberrations)	ILS 1	Negative	No induction of chromosome aberrations or polyploidy
		ILS 2	Negative	
PCS	Salmonella/E. coli (Mutation)	ILS 1	Negative	Not mutagenic
		ILS 2	Negative	
	MOLY (Mutation)	ILS 1	Negative	Not mutagenic
		ILS 2	Negative	
	HULY (Chromsome aberrations)	ILS 1	Negative	No induction of chromosome aberrations or polyploidy
		ILS 2	Negative	

WTR Toxicology Program: Summary of Results (GJH March 20, 2001)

Technology	Assay (Endpoint)	Exp.	Result	Conclusion
Analog	SCG (DNA Damage)	ILS 1 - 3hr	Positive (for TL only)	No DNA damage
		ILS 2 - 3hr	Negative	
		ILS 3 - 24hr	Negative	
	MN-BN (micronucleus)	ILS 1 - 3hr	Equivocal	Genotoxic
		ILS 2 - 3hr	Negative	
		ILS 3 - 24hr	Positive	
		ILS 4 - 24hr	Positive	
TDMA	SCG (DNA Damage)	ILS 1 - 3hr	Equivocal (for TL only)	No DNA damage
		ILS 2 - 24hr	Negative	
	MN-BN (micronucleus)	ILS 2	Negative	Genotoxic
		ILS 2 - 24hr	Positive	
		ILS 3 - 24hr	Positive	
CDMA	SCG (DNA Damage)	ILS 1 - 3hr	Equivocal	Inconclusive
		ILS 2 - 24hr	Negative	
	MN-BN (micronucleus)	ILS 1 - 3hr	Negative	Genotoxic
		ILS 2 - 24hr	Positive	

33

biological damage
chromosome damage.
micro

WTR Toxicology Program: Summary of Results (GJH March 20, 2001)

PCS	SCG (DNA Damage)	ILS 1 - 3hr	Negative	No DNA damage
		ILS 2 - 24hr	Negative	
	MN-BN (micronucleus)	ILS 1 - 3hr	Negative	Genotoxic
		ILS 2 - 24hr	Positive	

SCG In Vivo

Technology	Laboratory	Exp.	Endpoint	Result	Conclusion
Analog	ILS	1	% DNA	Negative	No DNA damage
			Tail Length	Negative	
			Tail Moment (Tice)	Negative	
		2	% DNA	Negative	
			Tail Length	Negative	
			Tail Moment (Tice)	Negative	
	UW	1	% DNA	Not reported	No DNA damage
			Tail Length	Equivocal	
			Tail Moment (Olive)	No analysis	
		2	% DNA	Not reported	
			Tail Length	Negative	
			Tail Moment (Olive)	No analysis	

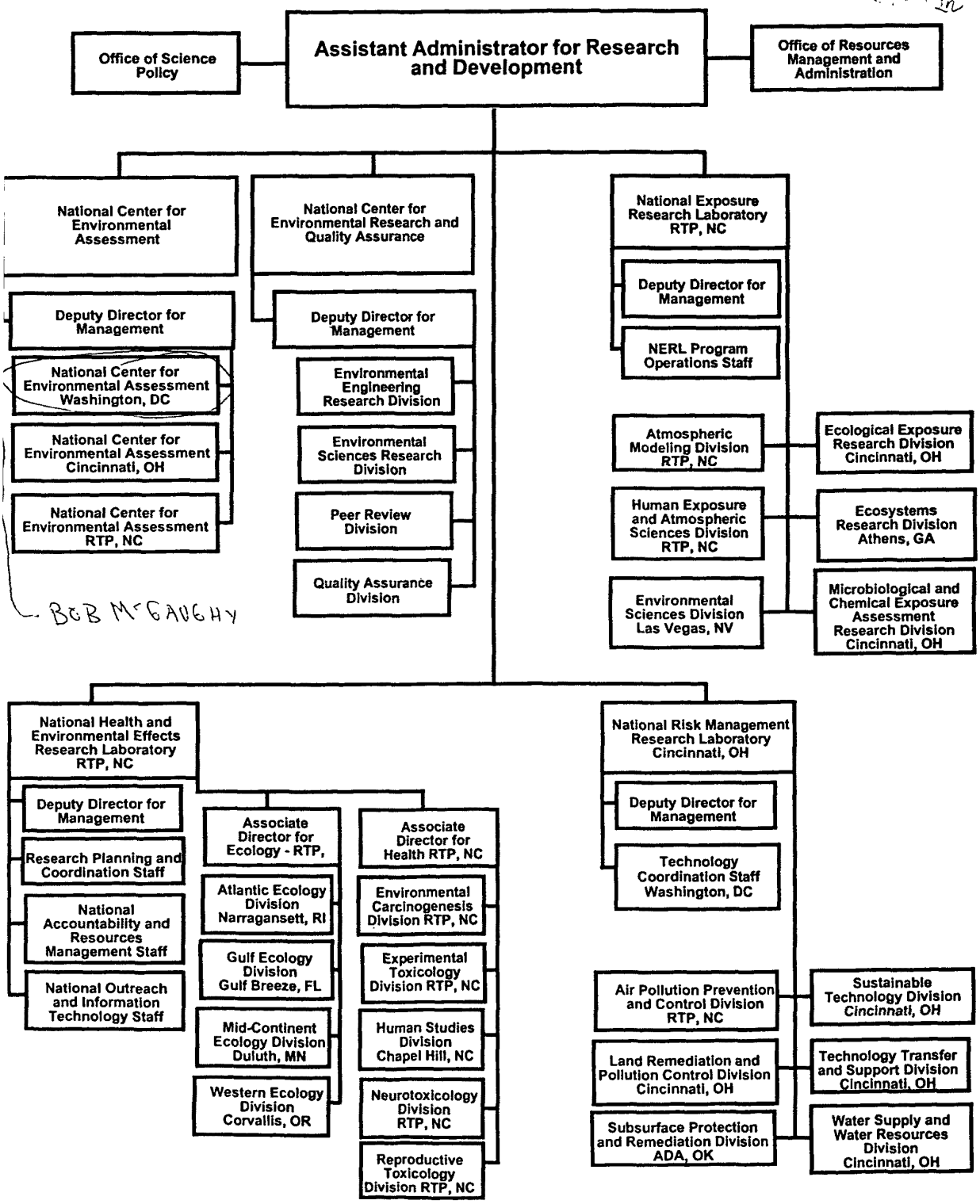
WTR Toxicology Program: Summary of Results (GJH March 20, 2001)

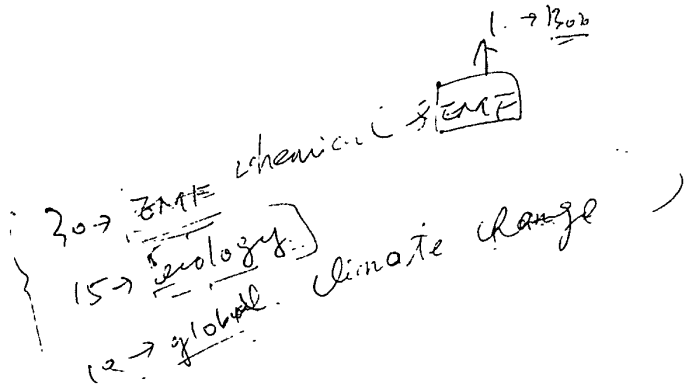
Technology	Laboratory	Exp.	Endpoint	Result	Conclusion
TDMA	ILS	1	% DNA	Negative	No DNA damage
			Tail Length	Negative	
			Tail Moment (Tice)	Negative	
		2	% DNA	Negative	
			Tail Length	Negative	
			Tail Moment (Tice)	Negative	
CDMA	ILS	1	% DNA	Negative	No DNA damage
			Tail Length	Negative	
			Tail Moment (Tice)	Negative	
		2	% DNA	Negative	
			Tail Length	Negative	
			Tail Moment (Tice)	Negative	
PCS	ILS	1	% DNA	Negative	No DNA damage
			Tail Length	Negative	
			Tail Moment (Tice)	Negative	
		2	% DNA	Negative	
			Tail Length	Negative	
			Tail Moment (Tice)	Negative	

附件五　美國環保署研發辦公室組織圖

Office of Research and Development

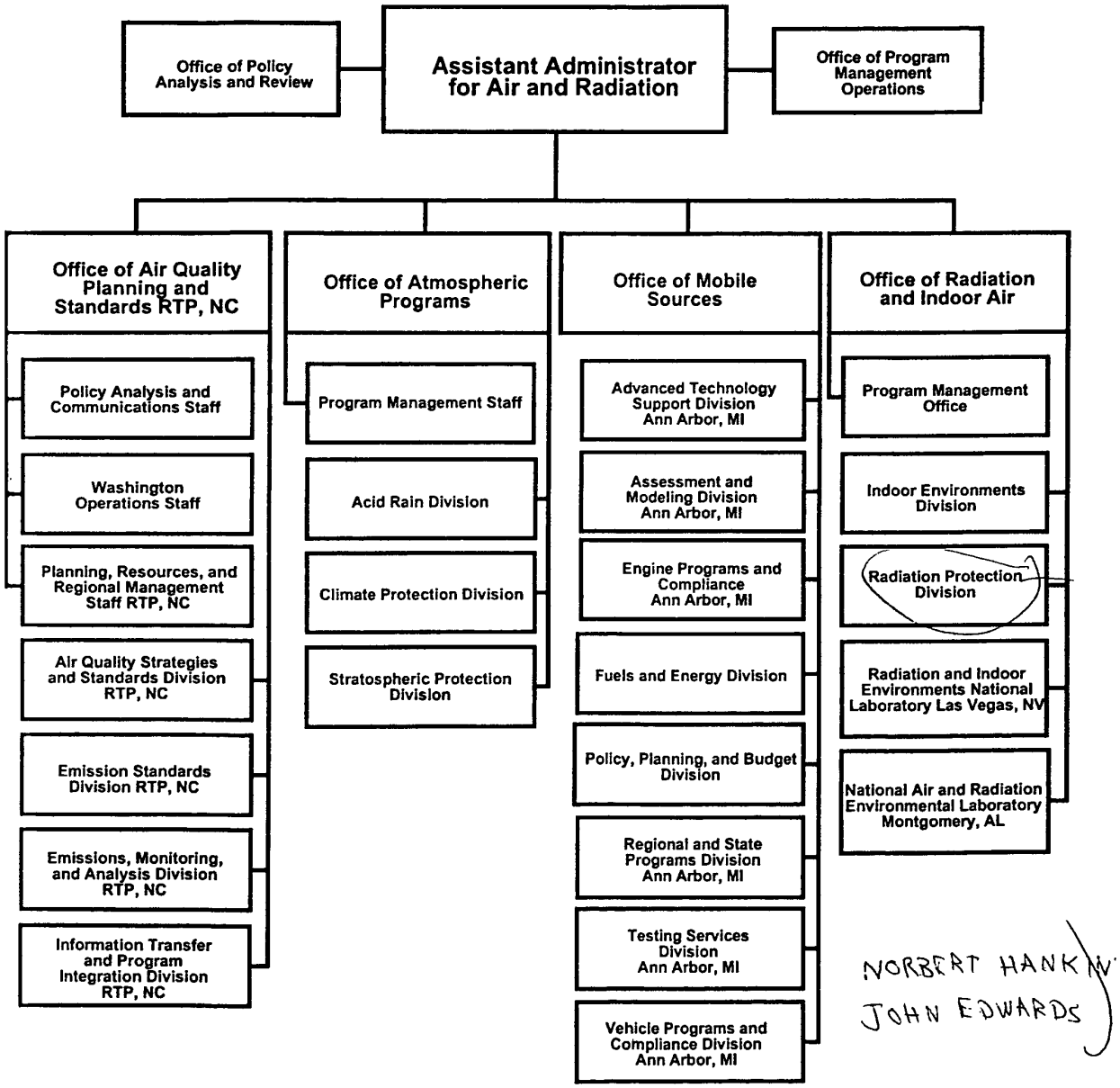
USEPA




1. → H_2O_2
EMF
chemical
30 → ozone
15 → ecology
12 → global climate change

附件六　美國環保署空氣及輻射辦公室組織圖

Office of Air and Radiation



18,000 employees.

8000

818

附件七　美國環保署空氣及輻射辦公室
愛德華博士簡報資料

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EPA
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Environmental Protection Agency

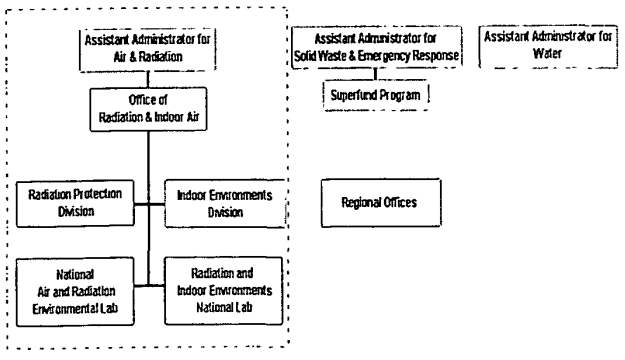
Radiation Protection Program

Briefing for International Visitors

March 2001

www.epa.gov/radiation

Organizational Framework



2

EMF

Las Vegas

Alabama

EPA's Niche in Radiation Protection

EPA has unique set of expertise for dealing with radiation in the environment

- **Regulation and policy development and implementation**
- **Science/technical information and studies**
- **Radioanalytical and policy support to other agency offices**
- **Emergency response**

3

Key Players and Stakeholders
Federal Agencies

- **EPA**
  - Issues standards to limit exposure
  - Measures radiation in environment and assesses effects on people and environment
  - Informs people about risks and promotes actions that reduce exposure
- **NRC**
  - Implements EPA's and its own standards
  - Regulates civilian uses of nuclear materials
- **DOE**
  - Manages materials from nuclear weapons production
- **HHS/FDA** - Center for Devices and Radiological Health
  - Key role in emergency response
  - Standards for x-ray machines and other products

4

Regulation and Policy Development and Implementation

- **Waste Management**
  - **Yucca Mountain, NV**
    - Fuel from power plants
    - EPA sets standard to be implemented by NRC & DOE
    - Proposal: August, 1999; Final: Summer, 2000
  - **Waste Isolation Pilot Project, NM**
    - Weapons waste
    - Certified safe operation of the facility, approves sites to ship waste to WIPP
  - **Low Activity Mixed Waste Rule**
    - Combined radioactive and hazardous wastes
    - To provide safe disposal options for waste generators

continued

Regulation and Policy Development and Implementation

(Continuation)

- **Waste Management** (cont'd)
  - **TENORM** - Technically Enhanced Naturally Occurring Radioactive Material
    - Assessing sources and risks
  - **MARSSIM** - Multi-Agency Radiation Survey and Site Investigation Manual
    - Guidance for radiation site surveys: planning, conducting, evaluating, and documenting
    - Training in Kazakhstan in September 2000
  - **MARLAP** - Multi-Agency Radiation Laboratory Protocols Manual
    - Guidance and framework for radioanalytical labs
    - Complements MARSSIM

continued

Regulation and Policy Development and Implementation

(Continuation)

- **Federal Guidance**
  - Sets principles and policies for other U.S. agencies to follow when developing their radiation policies
- **Air Standards**
  - Limit radioactive air emissions from government facilities, uranium mills, uranium mill tailings disposal piles, phosphogypsum
- **Capability Development**
  - Demonstrations, training and technology transfer to other U.S. Feds, Regions, States and tribes, and other countries
- **Public Information**

7

Science and Technical Information and Studies

- **Risk Assessment**
  - Methods /scientific bases for exposure, dose & risk assessments
  - Support for development of policy, guidance, and rule makings for radiation protection and risk management
- **Federal Guidance Technical Reports**
  - Provide current scientific and technical information
  - Used by regulators to assess dose and risk
- **BEIR VII** Biological Effects of Ionizing Radiation
  - Assessing viability of Linear No Threshold (LNT) hypothesis

continued

8

Science and Technical Information and Studies

(Continuation)

- **Environmental Radiation Ambient Monitoring System** (ERAMS)
  - National radiation monitoring network
  - 200+ stations
  - Monitors air, precipitation, drinking water and milk
- **Environmental Analysis**
  - Radiochemical and mixed waste analysis (supports Agency decisions at contaminated sites)
  - Lab and field measurement, sampling and QA
  - Mobile labs

9

drink water milk

Emergency Response

- **Preparation and Response**
  - Establish guidelines for protecting the public from radiation
  - Monitor and assess radioactivity in the environment
  - Coordinate the Federal response to an emergency if a nuclear accident occurs in a foreign country
- **Mobile Laboratories and Field Support**
  - Full radioanalytical capability
  - On site radioanalysis and assessment
  - Environmental monitoring and sampling at the site
- **Orphan Sources**
  - National program for identifying and managing uncontrolled sources

10

Radioanalytical and Policy Support to Other Agency Offices

- **Contaminated Site Clean Up**
  - Largest U.S. sites have radiation contamination
  - Provide technical support for radionuclide cleanups
    - Site sampling and analysis
    - Technology evaluation
- **Support to EPA Regions**
  - Decontamination and decommissioning (help assess environmental implications at nuclear power plants and fuel facilities)
  - Analytical data and technical assistance to support Agency decisions

11

Statutory Authorities for EPA's Radiation Program

- **Atomic Energy Act** (AEA)
  - Generally applicable standards: rad materials outside the fence
  - Federal Guidance: advise President on national rad protection guidance for Federal and State agencies
- **Nuclear Waste Policy Act** (NWPA) and **Amendments** (NWPAA)
  - Yucca Mountain: authority to set procedures for spent nuclear fuel and high level waste repositories
- **Waste Isolation Pilot Plan Land Withdrawal Act** (WIPP LWA)
  - WIPP oversight: regulatory authority over DOE

Continued

12

Statutory Authorities for EPA's Radiation Program

(Continuation)

- **Clean Water Act** (CWA) and **Safe Drinking Water Act** (SDWA)
  - Drinking water: mandate to protect current and future sources
  - Maximum Contaminant Levels (MCLs)
- **Comprehensive Environmental Response Compensation and Liability Act** (CERCLA)
  - Superfund: authority to clean up radioactively contaminated sites
- **Clean Air Act** (CAA)
  - National Emission Standards for Hazardous Air Pollutants: air emissions of radionuclides
- **ER Directives**
  - Several Federal-level directives defining our roles in emergencies

13

Non-Ionizing Program

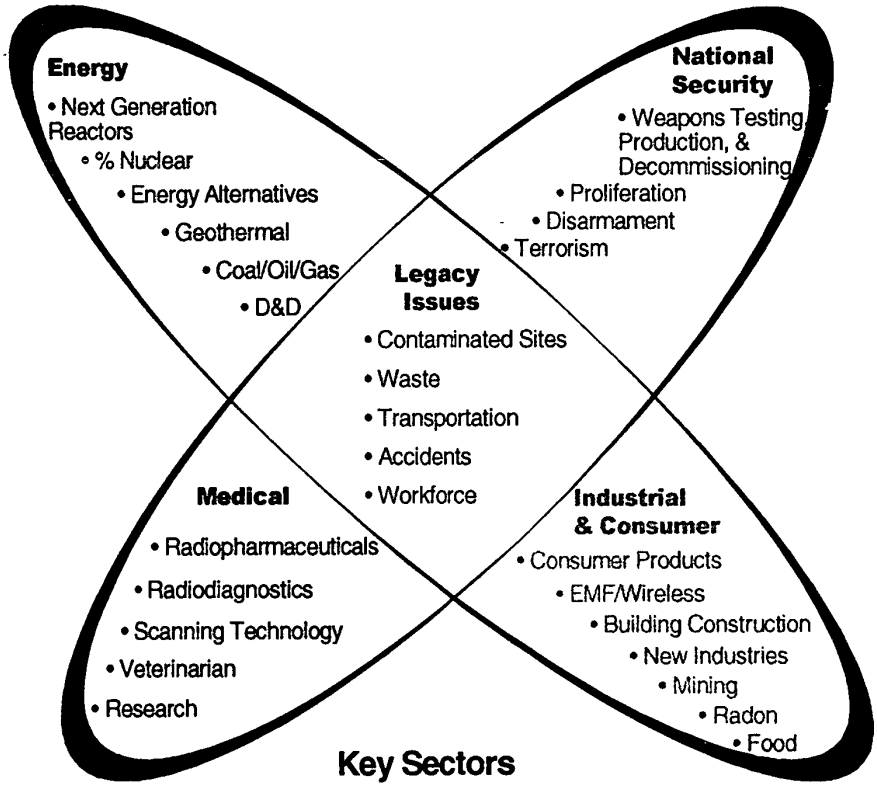
(Continuation)

- **Recently transferred to our program**
- **Strategy and Policy still being formed**
  - Science and Research
  - Policy
  - Analyses
  - Information and communications
- **Short-term, obvious role seems to be education and providing information**
  - Acknowledge and explain uncertainties
  - Explain possibilities of risk without undue alarm
  - Keep current on research
  - Advise people on how to scrutinized media reports and studies
  - Factsheets and webpages

14

APPENDIX B - KEY SECTORS WITH EXAMPLES OF CHALLENGES

The Future of Radiation Protection



PRINCIPLES FOR GUIDING ACTION
PROJECT ON THE FUTURE OF RADIATION PROTECTION

Pollution/Exposure Prevention

Pollution Prevention involves adopting practices that reduce at the source the amount of any hazardous substances or pollutants being released into the environment. It includes processes that eliminate the use of hazardous materials or increase the efficiency of their use. Exposure prevention involves adopting practices that reduce exposures to any hazardous substances that are released.

Pollution prevention approaches include substitution of materials, technology innovations, process modifications, redesign of products, improvements in training, and mass balance measurement to assess progress in reducing emissions. Exposure prevention includes inventory control, isolation and storage, and improvements in maintenance and housekeeping. Pollution/exposure prevention often saves money by reducing waste and health-related costs. Even where costs are substantial, it is justifiable to eliminate or reduce the use of hazardous materials and reduce exposures to them if the risks of damage to human health or the environment are high.

Public Right-to-Know

Right-to-Know involves assuring easy public (and public manager) access to complete and up-to-date information on the state of chemicals and radiation in the environment.

Actions to foster this principle include:

- Providing high quality, credible information;
- Filling in important information gaps with monitoring and research;
- Providing information in understandable, usable forms;
- Integrating information on chemical and radiation exposures into community-specific formats;
- Providing guidance to the public in interpreting data;
- Eliminating unnecessary secrecy;
- Integrating information on radiation into environmental databases;
- Integrating information from different Federal agencies.

Total Accounting

Total Accounting involves assessing the full cradle to grave costs and benefits of decisions, including impacts on human health and natural systems.

Challenges that arise in applying this principle include:

- Building agreement on methods;
- Doing life cycle analyses (cradle-to-grave, and cross-generational where appropriate);
- Valuing environmental resources and ecosystem services in doing environmental accounting;
- Assessing social costs to individuals and society as well as costs to the bottom line;
- Dealing with uncertainties and lack of data.

Risk Harmonization/ Cumulative Risk Assessment

This principle involves harmonizing approaches to radiation and chemicals based on a careful crosswalk between chemical and radiation models, parameters, risk calculations, and measurement techniques. It also requires a focus on understanding risks posed by cumulative exposures and interactions between hazardous agents.

Many of the major environmental risks we face require the simultaneous evaluation and control of both radiological and chemical risks, yet separation of the two persists along legal, regulatory, programmatic, training and operational lines. An additional complexity is the possible interaction between hazardous agents. Risk harmonization is necessary to allow us to evaluate cumulative risk and evolve beyond today's inadequate carcinogen by carcinogen approach to public health.

Inclusive Science

Inclusive Science involves bringing a wide range of disciplines and viewpoints to bear in research related to important issues of public policy.

Sound, rigorous scientific methods that can stand up to public and peer scrutiny are essential in all areas of research dealing with health and environmental risks. In many research areas related to public policy debates it is also essential to take an inclusive approach, drawing as appropriate on disciplines within the social sciences as well as the physical and biological sciences. Parties with views that are currently non-mainstream in character should have a role in the formulation of research agendas if their views are an important aspect of particular policy debates and their overall approach is evidence-oriented rather than ideological. Where apropos, an inclusive approach may employ alternative dispute resolution techniques to foster agreement on questions and methods for research.

Place-Based Tailoring

Place-based tailoring involves deliberate efforts to adapt policies to fit local or regional circumstances, and to encourage experimentation.

While uniform national policies and regulations are justified in many circumstances, they are sometimes adopted merely for bureaucratic convenience. As a result, "one size fits all" approaches sometimes fit no one. Place-based tailoring requires adopting a grass roots perspective as well as a national perspective. It also requires encouraging local and regional participation in the formulation of policies and regulations. Where appropriate, research can be tailored to address local questions, and information should be organized so that communities can look at local end exposures across media and disciplines.

Stewardship

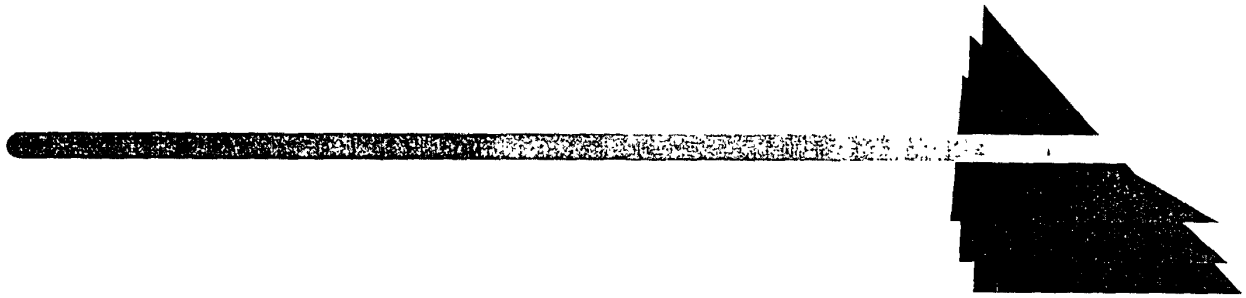
Stewardship involves taking responsibility for providing the expertise and resources to maintain across generations an adequate level of protection to human well being, health and the environment. Stewardship can be viewed as a "master principle" that encompasses all the others.

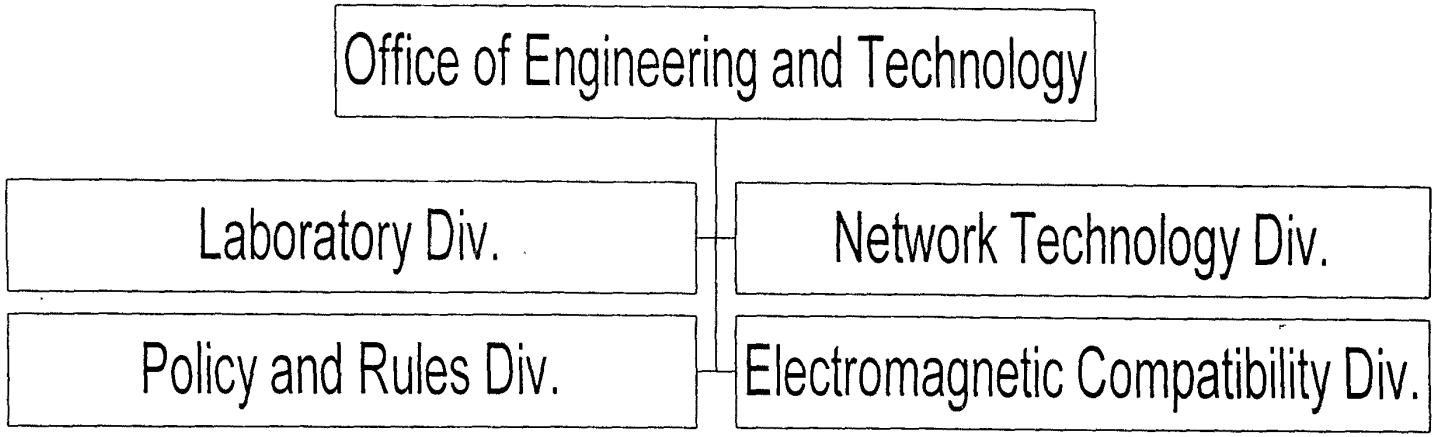
Stewardship is to hold something in trust for another. Historically, it was a means to protect a kingdom while the king was away or to govern for the sake of an underage king. Stewardship in today's context is willingness to choose service to the next generation over immediate self-interest. It is accepting accountability and providing leadership to assure the success of future generations. Stewardship is closely related to the concept of *sustainability*. Sustainable development is development that meets current needs without compromising the ability of future generations to meet their own needs.

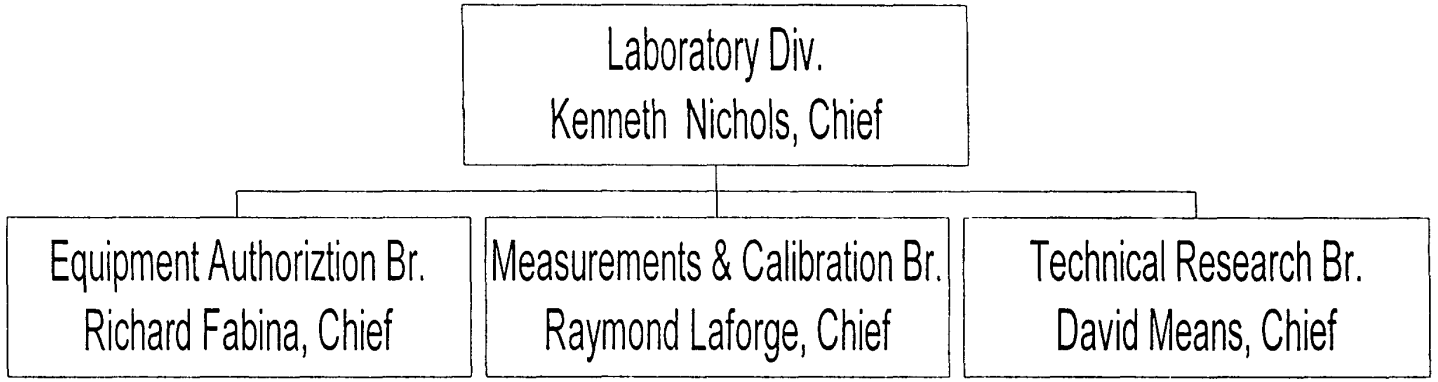
附件八　美國聯邦通訊委員會（FCC）
實驗室主任簡報資料

Welcome to the FCC Laboratory

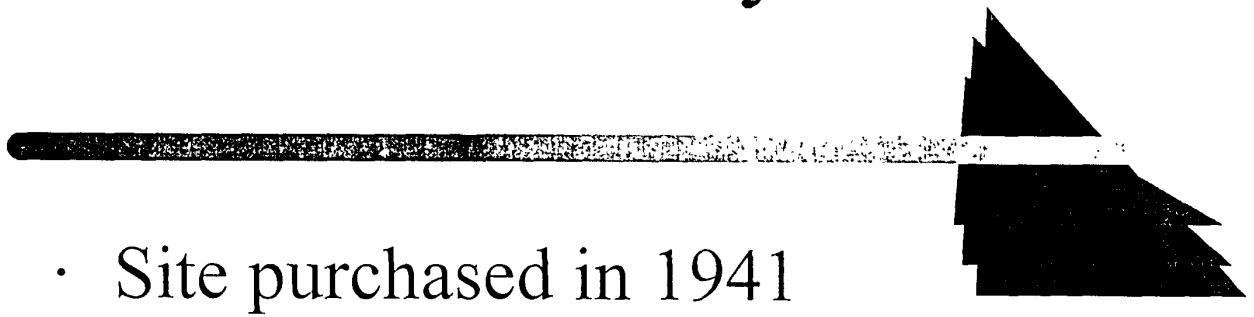
Columbia, MD




Office of Engineering and Technology
Laboratory Div.
Network Technology Div.
Policy and Rules Div.
Electromagnetic Compatibility Div.

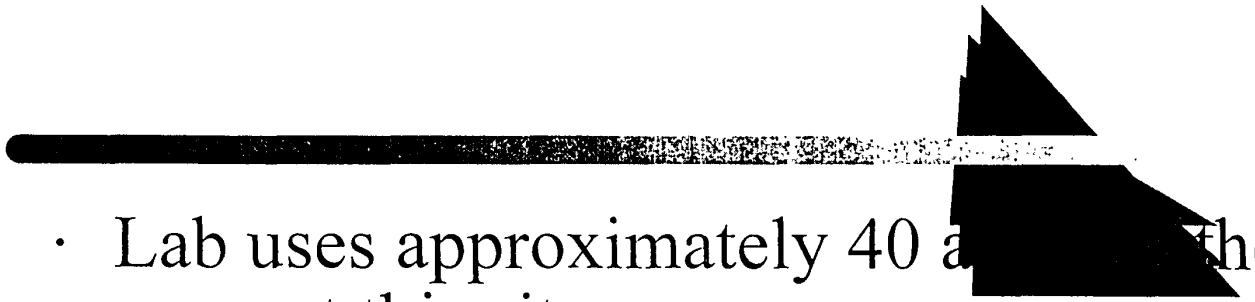

Laboratory Div.
Kenneth Nichols, Chief
Equipment Authoriztion Br.
Richard Fabina, Chief
Measurements & Calibration Br.
Raymond Laforge, Chief
Technical Research Br.
David Means, Chief

Laboratory Facilities



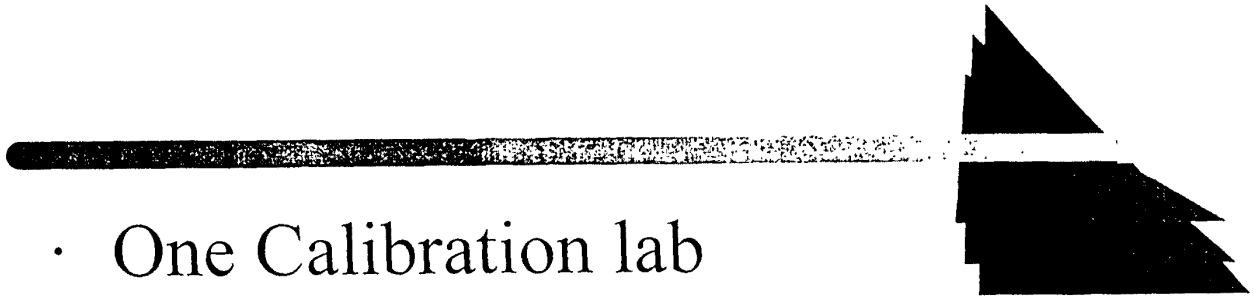
- Site purchased in 1941
- Presently staffed with 31 FTE
- Approximately $100 K operating budget

Laboratory Facilities cont.



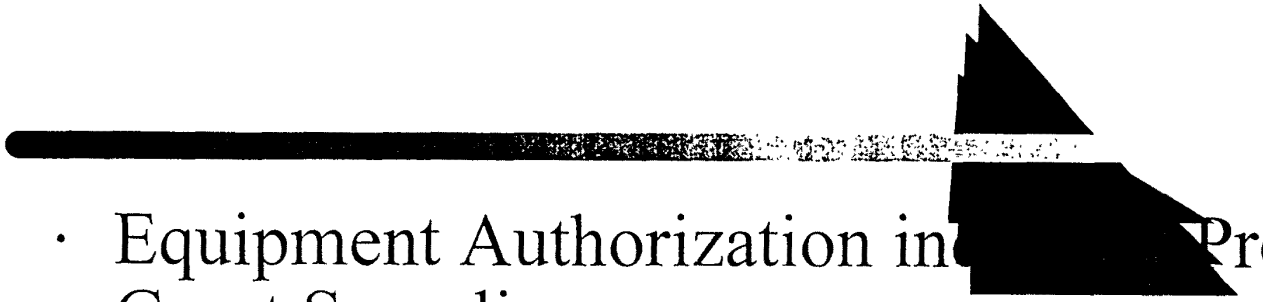
- Lab uses approximately 40 a[redacted]he 212 acres at this site
- Laboratory campus is composed of three buildings
  - 20,000 sq. ft. main building const. in 1974
  - Original office building is used for storage
  - Original Lab is used as maintenance shop

Laboratory Facilities cont.



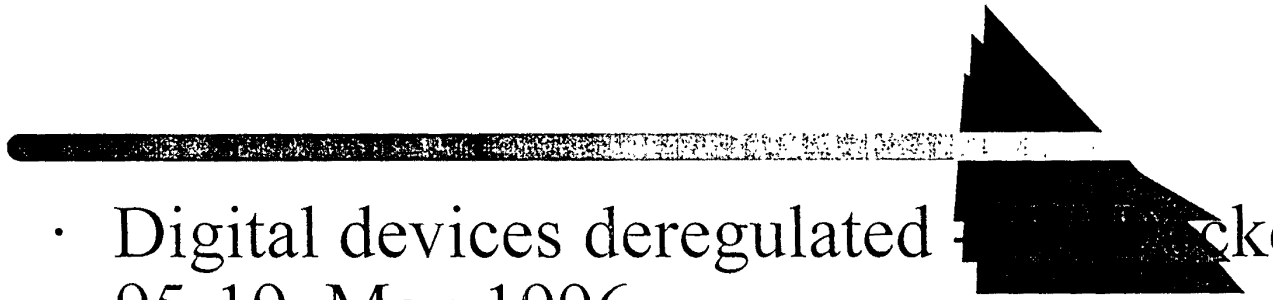
- One Calibration lab
- One TV lab
- Two indoor scanning labs
- One general purpose lab
  - GTEM cell and SAR measurement system
- Two screen rooms
- One Acoustic chamber
- One outdoor test site

Principal Functions



- Equipment Authorization in [redacted] Pre-Grant Sampling
- Post-Grant Sampling and Enforcement
- Technical Studies to establish new regulatory policies

Trends in Equip. Authorization



- Digital devices deregulated - [redacted]cket No. 95-19, May 1996
- Streamlining I, ET Docket No. 97-94, May 1998
- Streamlining II, GEN Docket No. 98-68, December 1998

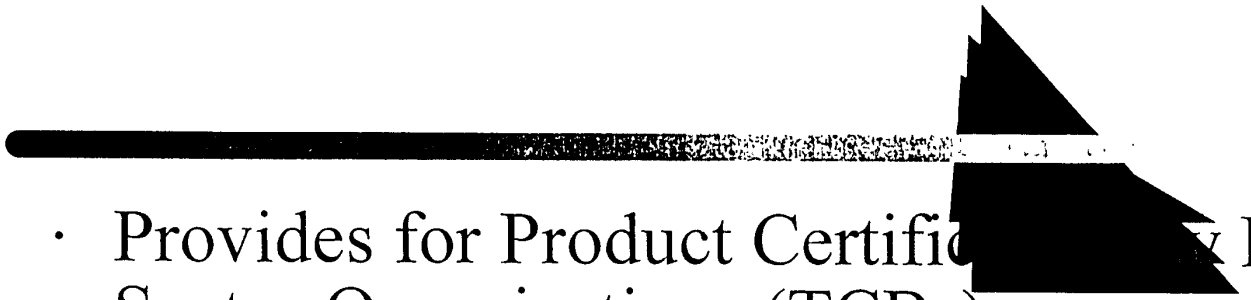
Digital Devices Deregulated

- Manufacturer's Self Declarat[redacted]s & Peripherals
- Must use Accredited Laboratories
- Provided easier approvals for Modular Computers

Streamlining I

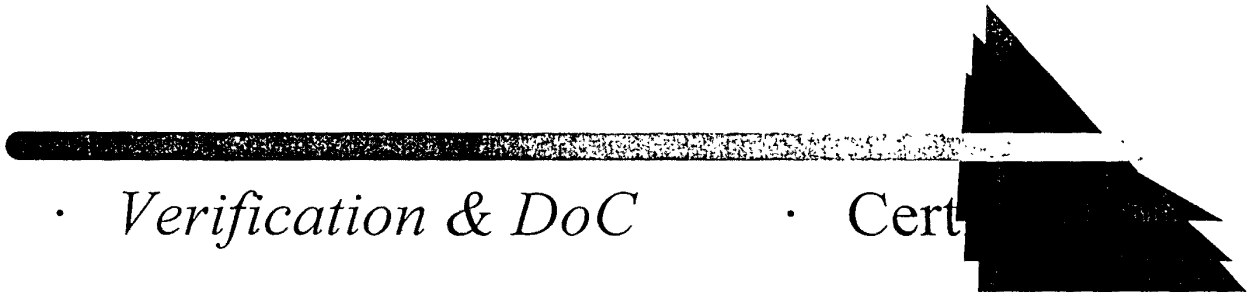
- Simplified Authorization Pr[redacted]
  - Replaced Multiple Processes with one process called Certification
  - Retained Self Authorization procedures
- Relaxed requirements for low-risk equipment
- Provided for Electronic Filing

Streamlining II



- Provides for Product Certifi[redacted] Private Sector Organizations (TCBs)
- Implements Mutual Recognition Agreements (MRAs)
- Establishes interim procedures for equipment approvals for Global Mobile Personal Communication for Satellite (GMPCS) terminals

Current Authorization Processes



- *Verification & DoC*
  - ☐ Self Approval
  - ☐ No Application filed with the FCC
- Cert
  - ☐ Unlicensed Devices & Licensed Transmitters
  - ☐ Application must be filed with FCC

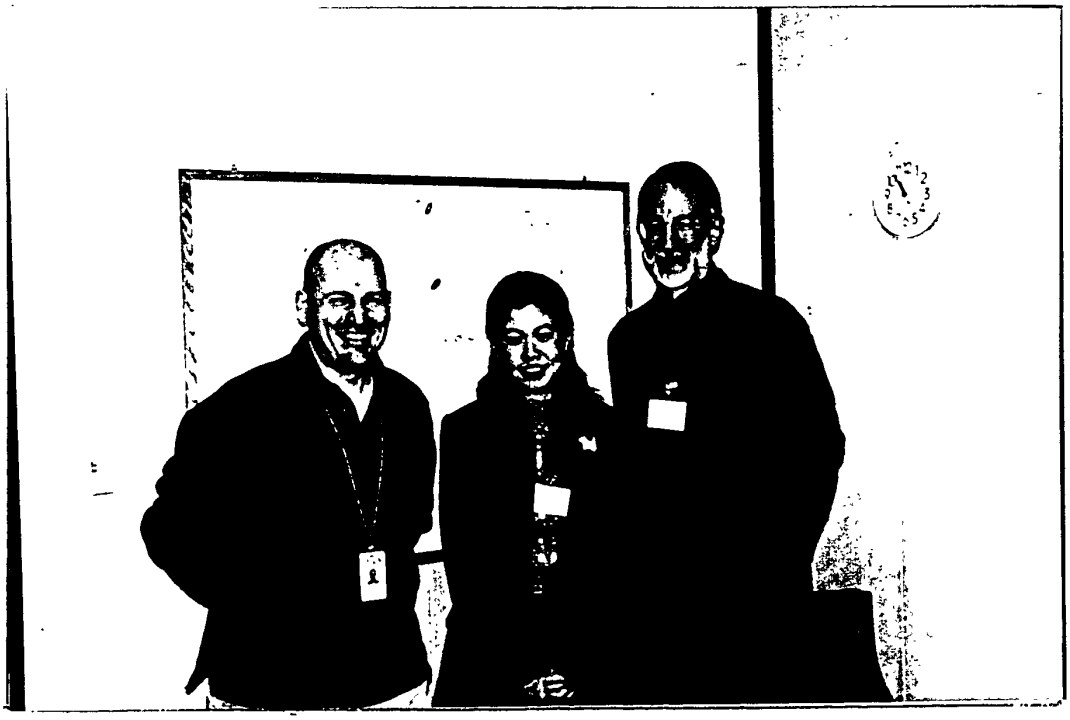
Technical Research Projects

- FM Broadcast Receiver Inte
  Susceptibility - Low Power FM BC
- Evaluation of First-Generation DTV Receivers
- Ultra-Wideband Devices
- RF Exposure (SAR)

研　習　活　動　照　片

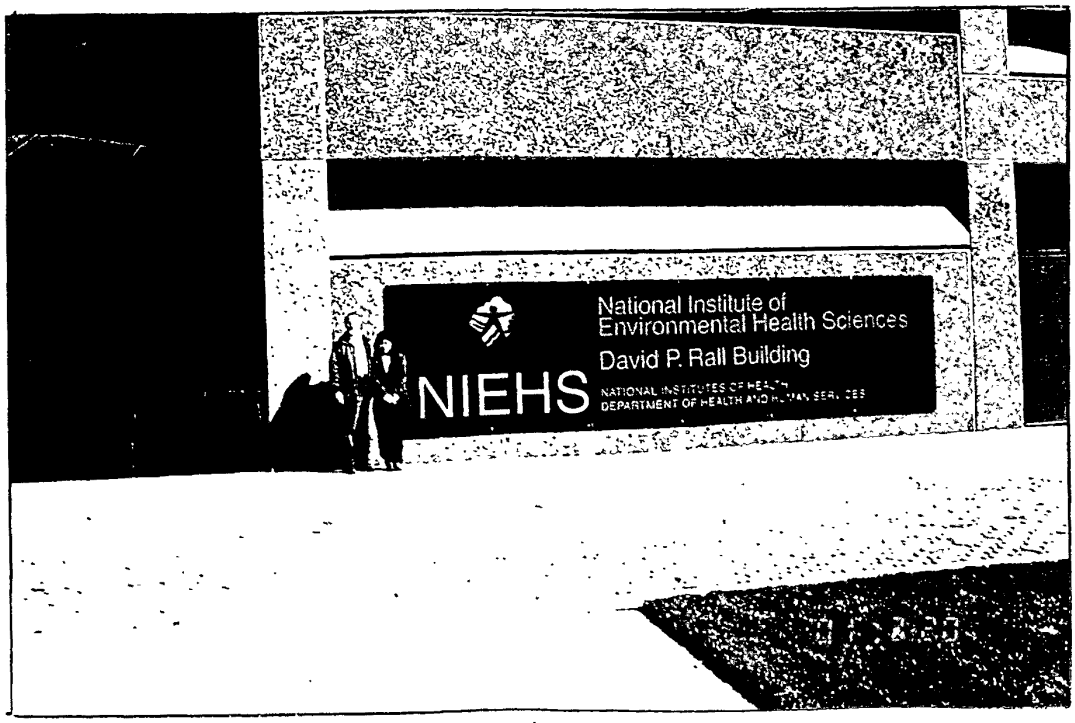
一、拜訪北卡州美國環保署所屬

RTP(Research Triangle Park)實驗室



二、拜訪北卡州整合系統公司（ILS）

三、至北卡州整合系統公司（ILS）實驗室參訪及研習



四、拜訪北卡州美國國家環境衛生科學研究所（NIEHS）

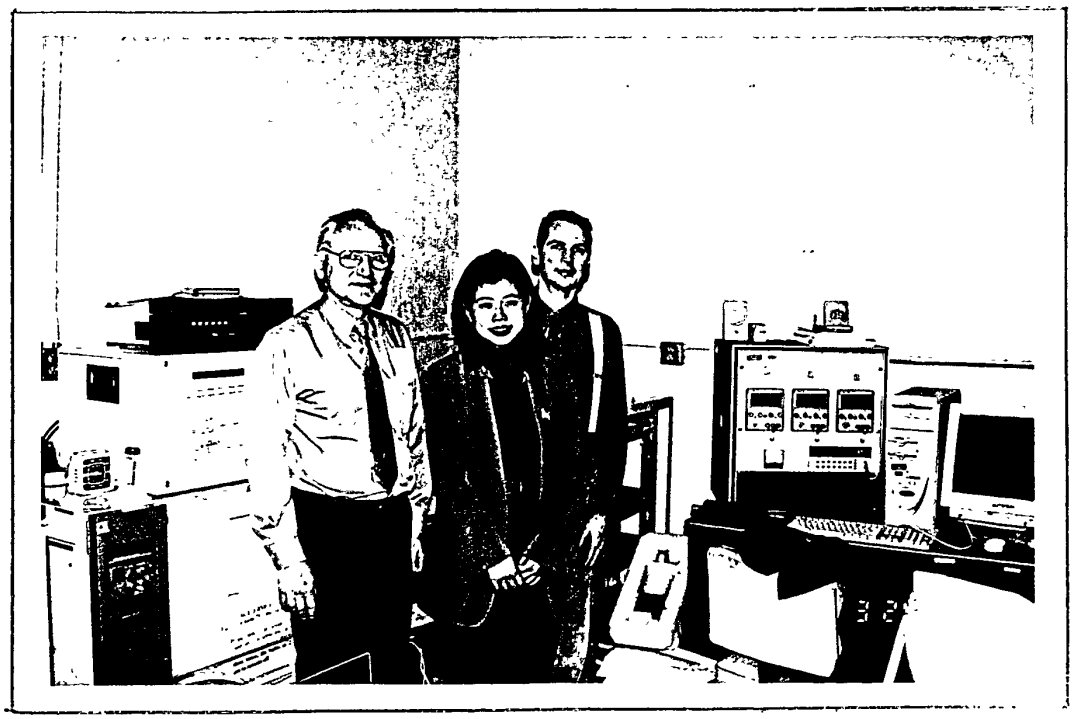
五、拜訪美國環保署總署

六、拜訪美國環保署輻射與空氣辦公室
（Office of Radiation and Indoor Air）

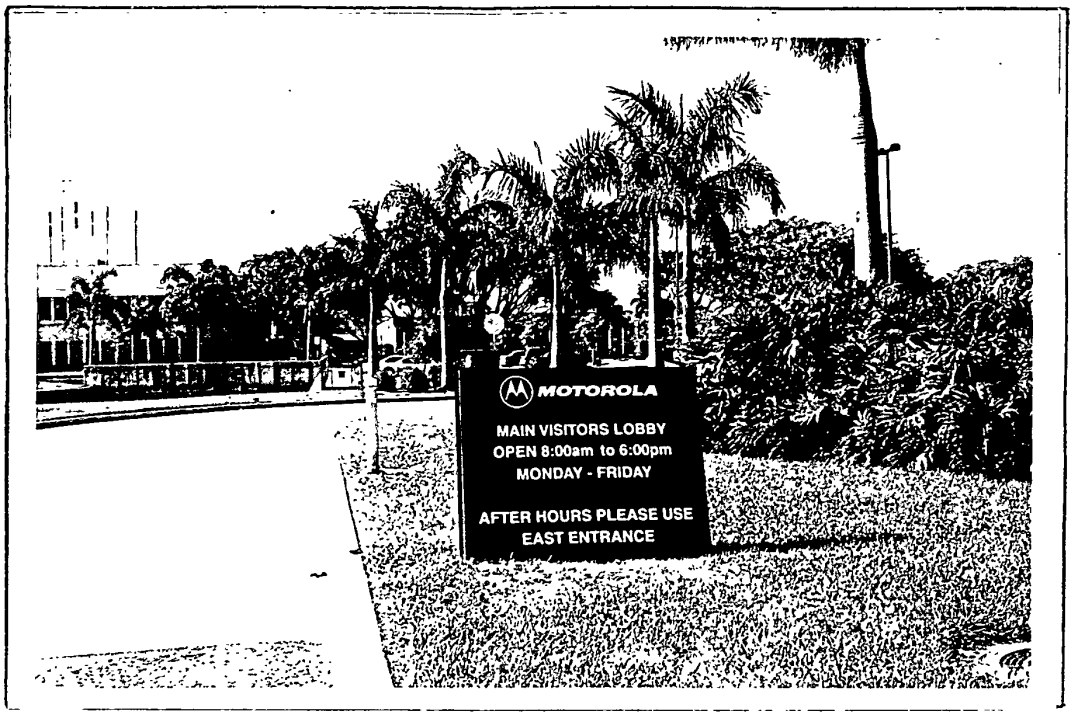
七、拜訪美國聯邦通訊委員會（FCC）總部

八、至美國聯邦通訊委員會實驗室參訪及研習

九、拜訪美國聯邦食品藥物管理局（FDA）總部



十、至美國聯邦食品藥物管理局參訪及研習



十一、拜訪美國佛羅里達州摩托蘿拉總公司

十二、拜訪美國佛羅里達州摩托蘿拉總公司