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饒連財　　徐士蘭
Lien T. Jao and S. L. Hsu

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饒 連 財　　徐 士 蘭

臺灣植物保護中心毒理組

（接受刊載日期：70年3月27日）

摘　　要

以含 0.5%食品抗氧化劑 BHA (Butylated hydroxyanisole) 或 BHT (Butylated hydroxytoluene) 之飼料餵小白鼠 (*Mus muselus*)，經 4 天之後再以胃管法 (Oral intubation) 注入陶斯松 (Chlorpyrifos) 或納乃得 (Methomyl) 等殺蟲劑，或經 2, 4 或 8 天後，以腹腔注射法 (Intra peritoneal injection) 將安眠藥（主成分爲 Pentobarbital）注入白鼠體內，研究此等抗氧化劑在動物體內對殺蟲劑及 Pentobarbital 藥效之影響。發現兩種抗氧化劑皆能增强白鼠對這些藥物之耐藥性 (Tolerance)。經 BHA 先處理 (Pretreatment) 4 天之鼠隻對納乃得較具耐藥性，其 LD_{50} 值爲對照組之 3.2 倍（雌鼠）及 2.2 倍（雄鼠），對 Pentobarital (80 mg/kg) 引起之昏睡時間縮短 33～47%（雌鼠）。經 BHT 先處理 4 天之鼠隻對陶斯松較具耐藥性，LD_{50} 值爲對照組之 2.1 倍（雌鼠）及 4.0 倍（雄鼠），對 Pentobarbital (80 mg/kg) 引起之昏睡時間縮短 54～67%，在本試驗中先處理的時間之長短雖對藥效有影響，但不甚大。雄鼠食用含 0.5% BHT 飼料達兩星期或更久時，呈現出好鬪及脫毛之現象。

* 臺灣植物保護中心毒理組研究報告第 25 號。

一、前　言

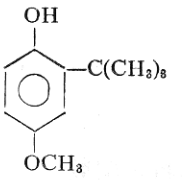
食品添加物及殘留在食物上之農藥係人類食品中極爲常見之外加物 (Foreign compounds)。各類農藥之使用乃保護作物及增產糧食所不可缺少的。而現代化之食品製造、加工、調配處理及貯存等過程中，爲了提高品質、增進風味、延長貯存期、減低腐敗之發生，許多食品添加物也被廣泛地使用。BHA 及 BHT 屬於人工合成之抗氧化劑，問世多年，用途甚廣，能增强許多物品之安定性 (Stability)，例如塑膠、合成及天然橡膠、汽油、潤滑油、蠟製品、油漆、油脂、動物飼料、合成樹脂、化粧品、口香糖、魚鮮（冷藏、鹽藏或乾品）等等[27]。

BHT 已被證明能够增强 DENA (Diethylnitrosamine) 致瘤腫[5,6] 以及 AAF (2-Acetylaminofluorence) 致癌性[19] 之作用，但對於 Aromatic amine derivatives[23], Polycyclic aromatic hydrocarbons[24,25] 以及 Azoxymethane[26] 等物質之致癌性却具有抑制效果。至於 BHT, BHA 對於農藥之毒理影響則迄未見報導。殘留在食物中之農藥與食品添加物如 BHA, BHT 等同時存在人體內彼此相互之間究將產生何種影響，乃成爲大家所關心與重視之問題。本實驗之目的卽在於對此問題作初步之探討。

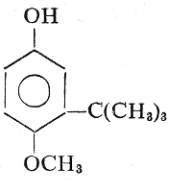
二、試驗材料與方法

本試驗所用之動物爲試驗室飼養之小白鼠 (*Mus muselus* ICR strain)。此品系爲 1974 年自臺北市美國海軍第二醫學研究所 (US Naval Medical Research Unit #2, Taipei) 引至臺灣植物保護中心繁殖。供試鼠隻年齡爲出生後 9～10 星期，體重 ♂ 爲 30±2 g，♀ 爲 25±2 g，雌雄分開處理。

抗氧化劑 (BHA, BHT) 購自振源化工原料公司（臺北市）純度皆在 99.7%以上，日本上野製藥株式會社之產品。BHA (Butylated hydroxyanisole) 係 2-tert-butyl-4-methoxyphenol (I) 與 3-tert-butyl-4-methoxyphenol (II) 之混合物。

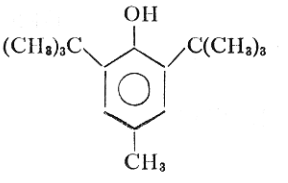


(I)



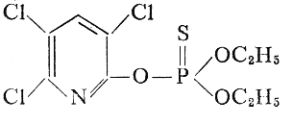
(II)

BHT (Butylated hydroxytoluene) 爲 2,6-di-tert-butyl-4-methylphenol

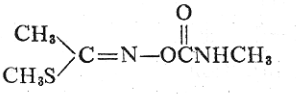


(一)殺蟲劑

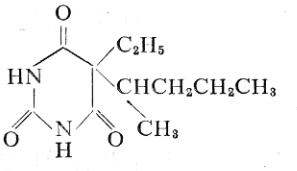
陶斯松 (Chlorpyrifos, Dursban), O, O-Diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate, 94%, Dows Chemical Co. 產品



納乃得 (Methomyl, Lannate), S-Methyl-N-(methyl-carbamoyloxy) thioacetamidate, 90% W. P. E. I. Du Pon de Nemours and Co. 產品



Nembutal (鈉鹽, Sodium salt) : pentobarbital in sterile solution, 50 mg/ml (Abbott Laboratories, Chicago, Ill. 60064).



(二)飼　料

約含 70%之玉米及 30%大豆（均爲粉粒狀），其中不含 BHA 或 BHT，臺糖新營副產品加工廠出品初生鷄飼料。

㈢丙酮及沙拉油

實驗使用之丙酮爲工業級再經蒸餾者，沙拉油則購自市面（統一牌）。

㈣ BHA 及 BHT 飼料之配製

先將 5 克的 BHA（或 BHT）溶於 250 ml 丙酮液中，再將之徐徐加至 1 公斤飼料中，同時不停地攪拌使飼料與藥液均勻混合，然後將此飼料在瓷盤上舖開，置放於陰涼通風處，待丙酮完全揮發後使用（約 24 小時）。

㈤殺蟲劑之處理

小白鼠雌雄分開，並以逢機取樣方式將之分成數組，分別以普通飼料、含 0.5% BHA 或 BHT 之飼料餵養。4 天後以特製之注射器將殺蟲劑（溶於沙拉油中）直接注入小白鼠胃中 (Oral intubation)，每隻接受 0.4 ml 之藥液。處理後放回試驗籠中以普通飼料飼養，觀察記錄 72 小時之死亡率。每一濃度至少重複三次，共 20 隻或以上，所得結果以統計分析法求出其 LD_{50} 及毒力迴歸線之斜率。

㈥ Nembutal 之處理

雌鼠分三組，每組 15 隻，分別餵以含 0.5% 抗氧化劑飼料及普通飼料。經 48, 96 或 192 小時後，自每一組中逢機取出 5 隻以腹腔注射 (Intraperitoneal injection) 方式將 Nembutal 注入老鼠體內，每隻 20 或 40 $\mu\ell$（含 Sodium pentobarbital 1 或 2 mg），觀察記錄其致昏迷所需之時間(Knock-down time) 及昏睡時間 (Sleeping time)。試驗重複三次，其結果以鄧肯氏多變距測驗法 (Duncan's multiple range test) 比較各處理間之差異。

三、結　果

㈠ BHA, BHT 對納乃得及陶斯松殺蟲劑毒效之影響

經過 BHA 或 BHT 先處理 (Pretreatment) 之小白鼠皆能對此兩種殺蟲劑產生較高之耐藥性 (Tolerance)（表一）。亦即其 LD_{50} 值增大 1.2 至 4.0 倍之多。兩種殺蟲劑中陶斯松屬於有機磷劑 (Organophosphate)，而納乃得則屬於氨基甲酸鹽劑(Carbamate)。其毒效作用機制(Mode of action)雖然相似，皆屬於抑制 Cholinesterase，然而由試驗結果可知兩種抗氧化劑對此兩種殺蟲劑在小白鼠體內之毒力影響則略有差異，即不論是雌鼠或雄鼠納乃得的毒力受到 BHA 之影響較 BHT 爲大，而陶斯松則正好相反。

表一　BHA 及 BHT 對納乃得與陶斯松殺蟲劑藥效之影響

Table 1. LD_{50} and slope values for Methomyl and Chlorpyrifos on mice as affected by BHA and BHT

預先處理 Pretreatment[a]	性別 Sex of mice	納乃得 Methomyl		陶斯松 Chlorpyrifos	
		LD_{50}[b]	斜率 Slope[c]	LD_{50}	斜率 Slope
對照組 Control	♀♀	40	5.1	135	3.9
0.5% BHA	♀♀	128	1.7	162	3.4
0.5% BHT	♀♀	72	2.6	287	2.6
對照組 Control	♂♂	29	7.0	78	3.6
0.5% BHA	♂♂	65	3.7	182	5.2
0.5% BHT	♂♂	44	3.7	310	5.3

a. Pretreatment: Animals were fed on chicken feed (70% corn plus 30% soybean in powder form) with or without 0.5% (W/W) antioxidant for 4 days before insecticide treatments.
b. LD_{50}: Milligrams per kilogram body weight, mg/kg.
c. Slope: Probits per unit log dosage.

正常而未接受先處理 (Pretreatment) 之小白鼠中，雌鼠之耐藥性遠較雄鼠爲高，但是經過 BHA 或 BHT 先處理後之雄鼠對陶斯松之耐藥性却超過經同樣處理之雌鼠。由於經 BHA 或 BHT 先處理過之鼠隻其耐藥性增加，而致藥效曲線之斜率 (Slope) 亦隨之降低，只有雄鼠對陶斯松例外。

㈡ BHA, BHT 對 Pentobarbital 藥效之影響

Nembutal 之主成分 Pentobarbital 乃具有使動物鎮靜及安眠之功效，該藥劑主要由肝臟分解代謝而排出體外[7,17]，並且已知對動物肝臟之藥物代謝酵素具有誘導作用 (Induction)[16,17,20]。由實驗結果（表二）知 BHA 及 BHT 在鼠體內能降

表二 BHA 及 BHT 對 Pentobarbital 藥效之影響

Table 2. Effects of BHA and BHT on the efficacy of pentobarbital in mice

預先處理（天）Pretreatment[a] days	藥量 Dosage mg/kg	昏迷數目 No. animal K. D.	致昏迷平均所需時間，分 Average time for[b] K. D., min.	平均昏睡時間，分 Average sleeping[c] time, min.
對照組 Control	40	10		
0.5% BHA 2	40	3		
4	40	7		
8	40	2		
0.5% BHT 2	40	3		
4	40	1		
8	40	1		
對照組 Control	80	15	7(4-11)	92(56-135)
0.5% BHA 2	80	15	8(4-12)	52(10-115)
4	80	15	10(4-19)	49(7- 74)
8	80	15	9(5-14)	61(21-130)
0.5% BHT 2	80	15	9(7-12)	42(23- 57)
4	80	15	9(5-19)	38(10- 60)
8	80	15	9(4-17)	30(17- 54)

a. Pretreatment: 15 animals of each were fed on chicken feed (70% corn plus 30% soybean in powder form) with or without 0.5% (W/W) antioxidant for 2, 4 or 8 days prior to the administration of pentobarbital.

b. Time for knock-down (K. D.) means the period of time between the injection of the drug and the appearance of knock-down phenomenon. An animal which had difficulty to stand up or walk firmly was considered to be knocked down. Values in parenthesis denote the range of K. D. time.

c. Sleeping time starts as soon as an animal is knocked down and ends as the animal is recovered and able to stand up or walk firmly. Values in parenthesis denote the range of sleeping time.

低 Pentobarbital 之藥效，尤其是 BHT。Pentobarbital 在低劑量時 (40 mg/kg) 使 BHT 先處理過之鼠隻僅有極少數產生昏迷現象，而對照組却有三分之二的鼠隻進入昏迷狀態。當 Pentobarbital 之劑量加倍至 80 mg/kg 時，全部試驗動物皆先後進入昏睡狀況。先經 BHA 及 BHT 處理過之鼠隻保持清醒的時間較對照組略微長些（約長 25～30%）。極爲明顯地可看出 BHA 與 BHT 皆對鼠隻之昏睡時間 (Sleeping time) 具有很突出之影響，而且 BHT 較 BHA 爲强。前處理 (Pretreatment) 的時間 (2, 4 或 8 天) 對此項影響雖略有不同，但其差異却不太大。0.5% BHA 能減少昏睡時間達 33～47%，而同樣濃度之 BHT 却能減少達 54～67%之多。前處理時間超過 8 天時昏睡時間未見有更進一步之縮短，反而鼠隻之體重略有下降之跡象，若達兩至三星期則食用含 0.5% BHT 飼料之鼠隻有呈現出好鬪及加速脫毛之現象。

四、討 論

BHA 及 BHT 在食品工業上被廣泛地當作抗氧化劑使用，吾人每天或多或少都吸食一些。迄今已有不少報告述及它們對動物體之影響，如行爲[18,22]、心臟[9]、肝臟及肝臟內之物質與酵素系統[2~4,8,10,12,14]。經 BHT 處理過之動物其肝臟內之 Smooth endoplasmic reticulum (SER) 顯著增加，藥物代謝酵素 (Microsomal drug-metabolizing enzymes) 活性被誘發 (induced) 增强[2,12~14]，BHA 則未被發現有類似功能[14]。本實驗結果顯示 BHT 與 BHA 皆有降低納乃得及陶斯松殺蟲劑之毒力以及 Pentobarbital 藥效之作用，亦卽經 BHA

或 BHT 先處理過之鼠隻對殺蟲劑及 Pentobarbital 之耐藥性增强。此種現象之產生可能係由於Microsomal drug-metabolizing enzymes 之活性受到誘導 (Induction) 而增加之故。至於能被 BHT 與 BHA 所誘增之酵素系統未必完全一致，此可由表一之結果可看出。對 Pentobarital 及有機磷劑陶斯松代謝酵素系統而言，BHT 之誘增能力遠較 BHA 爲大，而對氨基甲酸鹽系的納乃得代謝酵素系統則正好相反。至於此兩種抗氧化劑對其他的有機磷及氨基甲酸鹽殺蟲劑是否分別有類似傾向之影響，則有待進一步證實。

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Toxicological Effects of Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) on Insecticide Chemicals

I. Effects of BHA and BHT on Chlorpyrifos, Methomyl and Pentobarbital

Lien T. Jao and S. L. Hsu

Abstract

BHA (Butylated hydroxyanisole) and BHT (Butylated hydroxytoluene) are antioxidants extensively utilized in modern food processings. Mice (*Mus muselus*) of both sexes pretreated with antioxidants (0.5% by weight in chicken feed mainly composed of powdered corn and soybean) for 2, 4 or 8 days were used to test the effect of these antioxidants on the toxicity of Chlorpyrifos and Methomyl insecticides and the effectiveness of pentobarbital, the active ingredient of the sedative Nembutal. Evidence shows that both BHA and BHT have a significant effect on both sexes of the animal tested in drug-metabolizing ability. Mice pretreated with BHA for four days are more tolerant to Methomyl with LD_{50} values being 3.2 (♀♀) and 2.2 (♂♂) times as high as those of the control groups. This pretreatment also reduces the pentobarbital induced sleeping time by 33 to 47% (♀♀). However, animals pretreated with BHT for the same period show higher tolerance to Chlorpyrifos with LD_{50} values being 2.1 (♀♀) and 4.0 (♂♂) times as high as those of the control groups. It also reduced the sleeping time by 54 to 67%. Male mice raised on 0.5% BHT feed for two weeks or longer show a tendency of becoming more aggressive and a phenomenon of hair falling out. The mechanism of these effects is discussed.