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研究簡報

殺菌劑貝芬替(Carbendazim)對鼠致胚胎畸形性評估

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貝芬替爲氨基甲酸鹽類殺菌劑，是免賴得(benomyl)代謝過程中主要產物[4]。往昔研究已對免賴得之生殖毒性及致畸形性多所探討[1,5,6,9,12]，至於貝芬替對雄鼠生殖毒性亦有部份研究，尤其它可造成雄鼠精細胞結痂、微細微管蛋白中毒、輸精管閉鎖及睪丸萎縮[10,11]。唯貝芬替對致動物胚胎畸形性等現象則尚無報告。本研究之重點即在探討不同劑量貝芬替之致畸胎性。預期此研究結果可對免賴得及貝芬替兩種殺菌劑提供安全評估及管理之參考依據。

本試驗供試農藥貝芬替(methyl-2-benzimidazole carbamate)99 %原體，由興農農藥公司提供。投藥時，各劑量以聚乙烯甘醇(polyethylene glycol 200, PEG 200)溶解配製。供試動物爲SPF Wistar品系鼠(*Rattus norvegicus*)購自國立成功大學醫學院動物中心，4週齡(雌: 70 ± 8g，雄: 80 ± 10g)，於本所SPF動物房飼養至10週齡(雄: 334 ± 26g，雌: 250 ± 18g)。動物房飼育環境: 溫度22 ± 2℃，相對濕度50-70 %，空氣循環爲15-20次/時，每日光照12小時，飼料爲Purina Laboratory Chow，充分供應飲水與任食。

將貝芬替溶於PEG 200中，試藥配製以全部試驗母鼠第7天之平均體重(約爲270g)爲基準，每隻母鼠投予1ml，往後隨每日體重增減而調整投予量，分別設20、40、60、80 mg/kg/day之劑量組，慰劑組僅投予PEG 200以及另設未處理之對照組。雌鼠之配種，於每個飼育盒(polycarbonate,Clea Japan, 長265mm × 寬425mm × 高200mm)置雌、雄各乙隻，每日早晨檢視膣道栓塞(vaginal plug)，出現時視爲懷孕第0天。懷孕雌鼠逢機分配至各處理組，懷孕第6至15天每日以胃管投予藥劑。第20天時以乙醚昏迷，剖腹取出子宮秤重，同時檢視死活胎仔數、畸胎數與畸形情形、黃體數、被重吸收數、著床數、子鼠性別、並秤胎仔體重與母鼠肝重與體重比。劑量範圍選擇試驗時，劑量分別爲100、200、

300、500 mg/kg/day，藥劑投予時間爲懷孕第7至20天，於懷孕第21天解剖。黃體數、著床數、子鼠數、母鼠與子鼠體重、肝臟與體重比、胎盤重、性比率及胚胎早期死亡等之統計方法均以 t-test 進行；進行處理組對對照組比較前，對照組先對未處理組進行 t-test，經測試結果，兩者差異不顯著。

劑量範圍選擇試驗時，以貝芬替經由胃管法投予母鼠 100、200、300及500 mg/kg/day 劑量後之各項觀察如表一，結果顯示，各劑量於試驗期內均無母鼠死亡，各劑量處理組母鼠黃體數及著床數與對照組無顯著差異，由於各劑量處理組大部份子鼠於胚胎形成時早期死亡，致無法比較胚胎數。表二所列之結果爲貝芬替對胚胎發育及子鼠的影響。貝芬替對著床前胚損失的影響不顯著，對著床後胚胎死亡或被重吸收數，則隨劑量增加有遞增之趨勢，被重吸收數大於或等於3之窩數各試驗組均較對照組高。至於試驗期間母鼠之增重，各處理組均較對照組顯著減少；母鼠肝臟與體重比，在所有劑量組均顯著大於對照組；胚胎早期死亡數，各劑量處理組均顯著高於對照組；100 mg/kg/day 劑量組出生之8隻仔鼠體重遠較對照組者輕，其餘劑量因胚胎早期死亡無子鼠出生。根據劑量範圍選擇試驗結果，正式試驗劑量設爲20、40、60、80 mg/kg/day。

表三所列爲實驗組不同劑量之貝芬替經由胃管法投予母鼠後之各項觀察；各劑量處理組於試驗期內均無母鼠死亡，各劑量處理組對母鼠黃體數與著床數均無顯著影響，除80mg/kg/day劑量因大部份子鼠於胚胎形成時早期死亡，無法作比較外，其餘劑量均無子鼠死亡。表四所列之結果爲貝芬替對胚胎發育及子鼠的影響，貝芬替對著床前胚損失的影響不明顯，對著床後胚胎死亡或被重吸收數，則隨劑量增加而遞增，被重吸收數大於或等於3之窩數亦隨劑量增加而上升。試驗期間母鼠之增重，各處理組均較對照組顯著減少；母鼠肝臟/體重比，在60與80mg/kg/day劑量處理時顯著大於對照組；子鼠性比率方面，除80 mg/kg/day劑量組外，其它劑量組之比值均與對照組相似。貝芬替對胎仔發育性狀之影響如表五與表六；80mg/kg/day劑量組僅2隻子鼠存活外，其餘劑量均無死亡子鼠，子鼠體重與胎盤重均隨著劑量增加而遞減；子鼠外觀於40mg/kg /day (圖一 A) 與60mg/kg/day出現露腦畸形 (圖一 A)，60mg/kg/day亦出現無尾巴與尾巴捲曲情形 (圖一 B)。藥劑之致畸胎性試驗會因藥劑種類、投藥劑量以及懷

表一、劑量範圍試驗之貝芬替對大鼠出生前之毒性

Table 1. Prenatal toxicity of carbendazim exposure on pregnant rats in the range finding test

Observations	control	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day	500 mg/kg/day
No. pregnant	9	5	5	5	4
No. corpus lutea/litter	22.1±3.8	23.0±5.8	21.6±3.9	20.6±5.4	20.5±4.7
No. implantation/litter	13.0±1.7	14.2±2.2	14.0±1.2	12.8±2.2	14.5±1.7
No. fetus/litter	12.1±1.3	_[1)]	_	_	_
No. dead fetus	0	8	_	_	_
No. litter with≧3 dead fetuses	0	2	_	_	_

[1)] Embryolethality occurred during the organogenesis and fetal growth stage.

表二、口服貝芬替之劑量範圍試驗對母鼠與子鼠之影響

Table 2. Developmental effects of oral administration of carbendazim on maternal and fetal rats in the range finding test

Observations	control	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day	500 mg/kg/day
Maternal					
No. litter	9	5	5	4	5
No. preimplantation loss	2	46	38	39	57
average	9.1	9.2	7.6	9.8	11.4
No. post implantation loss					
early	8	26	29	27	15
late	0	42	41	37	43
total	8	68	70	64	58
average No. loss/ litter	0.9	13.6	14.0	12.8	14.5
No. litter with ≧3 resorptions	1	5	5	5	4
weight gain, g	56.8±9.3	33.7±9.3*	40.7±10.6*	45.4±20.0	31.3±5.0*
liver weight/body weight	0.039±0.003	0.049±0.002*	0.053±0.004*	0.055±0.001*	0.056±0.005*
Fetal					
sex ratio of fetus (male/female)	1.2±0.4	3.0±1.4*	_2)	_	_
No. of embryolethality	0.9±1.1	12.6±4.1*	14.0±1.2*	12.8±2.2*	14.5±1.7*
B.W. of fetus, g	6.0±0.5	3.6±0.5	_	_	_

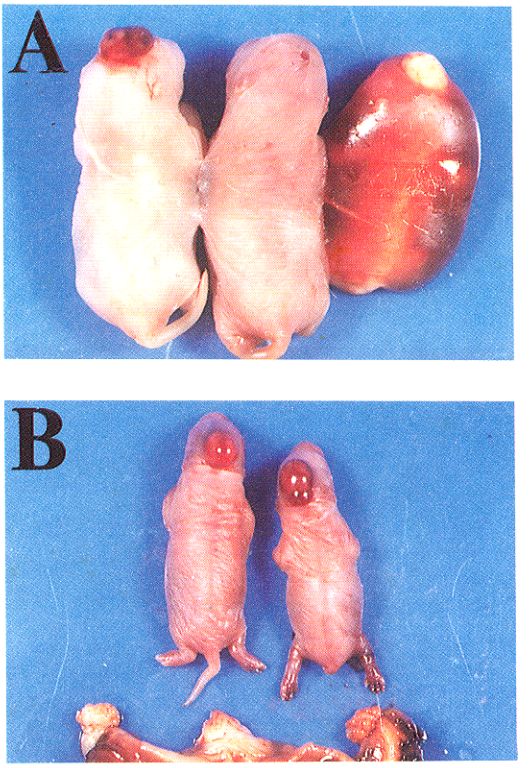
1) Means of the treatment followed by ` * ´ is significantly different from that of control at p = 0.05(t test).

2) Embryolethality occurred during the organogenesis and fetal growth stage.

孕時期而導致不同結果[8]。

由於貝芬替爲免賴得代謝產物之一，而免賴得可引起致畸胎之劑量主要在200至300 mg/kg/day之範圍內[1]。已知免賴得在鼠及狗體內代謝後主要產物有methyl 5-hydroxy-2-benzimidazole carbamate、carbendazim及methy 4-hydroxy-2-benzimidazole carbamate[7]，本研究以貝芬替投予大鼠後產生露腦畸形現象與免賴得所產生之畸形相同，因此推論貝芬替可能爲免賴得之主要致畸胎成分，至於其它代謝產物對畸形性之影響程度有待進一步探討。

貝芬替與免賴得均爲本省大量使用之廣效性殺菌劑，據1993年本省農藥成品產銷統計記錄，免賴得成品本省年銷售量爲185噸，貝芬替成品爲83噸左右[2]，使用量及使用範圍均甚廣，根據本所年實際測得貝芬替於蔬果之殘留量估算，理論最大殘留值爲0.00764 mg/kg，及實際取食殘留值0.00031 mg/kg[3]，依本試驗無作用劑量(NOEL)20 mg/kg/day，推估孕婦之ADI值爲0.2 mg/kg/day，而以安全係數1000計，ADI值爲0.02mg/kg/day，據此於安全係數100與1000計算理論最大殘留值佔ADI值分別爲3.82％與38.2％，而實際取食殘留值佔ADI值爲0.16％與1.6％。由此可知，貝芬替



圖一、貝芬替處理後鼠胎出現(A)露腦畸形(B)尾巴彎曲與無尾畸形。

Fig 1. (A) Exencephaly and (B) bent tail and tail absence in fetus from carbendazim-treated rat.

表三、貝芬替對懷孕鼠生產前之毒性

Table 3. Prenatal toxicity of carbendazim exposure on pregnant rats

Observations	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	80 mg/kg/day
No. pregnant	9	6	6	6	6
No. corpus lutea/litter	22.6±7.5	21.5±1.9	21.2±6.7	23.0±4.4	21.3±7.3
No. implantation/litter	13.8±3.0	15.3±1.0	12.8±1.2	12.8±2.4	12.2±4.0
No. fetus/litter	12.4±3.2	13.3±2.1	11.0±1.4	7.7±4.9*	_[2]

[1] Means of the treatment followed by `*´ is significantly different from that of the control at p=0.05(t test).

[2] Embryolethality occurred during the organogenesis and fetal growth stage.

表四、口服貝芬替試驗組對母鼠與子鼠之影響

Table 4. Developmental effects of oral administration of carbendazim on maternal and fetal rats in the test

Obsrvations	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	80 mg/kg/day
Maternal					
No. litter	9	6	5	6	5
No. preimplantation loss	79	37	54	61	55
average	8.8	6.2	10.8	10.2	11.0
No. post implantation loss					
early	12	8	6	1	29
late	0	4	5	30	42
total	12	12	11	31	71
average No. loss/ litter	2.4	2.4	2.8	7.8	11.8
No. litter with ≧3 resorptions	1	2	3	4	6
weight gain, g	66.7±10.3	44.7±16.9*	41.5±11.8*	44.2±12.8*	39.7±18.8*
liver weight/body weight	0.041±0.004	0.038±0.002	0.043±0.002	0.045±0.001*	0.047±0.006*
Fetal					
sex ratio of fetus (male/female)	0.8±0.3	1.0±0.6	1.2±0.9	1.1±0.6	_[2)]

[1)] Means of the treatment followed by `*´ is significantly different from that of control at p = 0.05(t test).

[2)] Embryolethality occurred during the organogenesis and fetal growth stage.

表五、貝芬替試驗組誘發之子鼠存活率、體重及胎盤重

Table 5. Fetal survival rate and malformation induced by prenatal carbendazim exposure in the test

Observations	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	80 mg/kg/day
No. fetus	112	80	66	46	2
body weight, g	4.6±0.40	4.2±0.40*	3.2±0.50*	3.0±0.50*	2.3±0.01*
placental weight, g	0.71±0.11	0.56±0.09*	0.55±0.09*	0.50±0.13*	0.43±0.08*

1) Means of the treatment followed by ` * ´ is significantly different from that of the control at p=0.05(t test).

表六、貝芬替試驗組誘發之子鼠外觀異常現象

Table 6. Fetal malformation induced by prenatal carbendazim exposure in the test

Observations	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	80 mg/kg/day
No. fetus with extermally congentital anomalies exencephaly					
mild	0	0	5	11	0
moderate	0	0	1	0	0
severe	0	0	0	1	0
tail absent	0	0	0	1	0
bent tail	0	0	0	1	0
cleft palate	0	0	0	0	1
total	0	0	6	14	1

對懷孕婦女在致畸形風險上應屬安全。

(關鍵詞 ： 貝芬替、鼠、致胚胎畸形性)

謝　　辭

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ABSTRACT

Lu, S.Y., Lin, H.W., Wang, S.C. 1995. Evaluation on teratogenicity of carbendazim in rats. Plant Prot. Bull. 37: 331-338. (Taiwan Agricultural Chemicals and Toxic Substance Research Institute, 11 Kuang Ming Road, Wufeng, Taichung, Taiwan 413, Republic of China)

The objective of this study is to evaluate the teratogenic potential of carbendazim Results showed that there is no significant difference in the number of corpus lutea, implantations and preimplantation loss between treated and control group. Doses above 100 mg/kg /day caused 100 % mortality in fetus and embryolethality. Doses were administered to rats from the 6th day after gestation through the 15th. No effects on the number of corpus luteal, implantations and preimplantation loss was found in treated rats. The number of fetus with 80 mg/kg/day result in embryolethality. Although the number of fetus was less than control in 60 mg/kg/day both the number of postimplantation loss (or resorptions) and resorptions more or equal than 3 in all treated groups were not significantly different from that of control. The body weight gain in dam dropped down in all treated groups. Liver weight per body weight in treated dam is more heavy than that of control exception of dose 20 mg/kg/day. Sex ratio in fetus have no significant difference between treated and control with the exception of dose 80 mg/kg/day. No dead fetus occurred in treated and control groups. Birth and placental weight exhibited decrease in all treated groups. Malformations as exencephaly, tail absence, bent tail and cleft palate appears after dosing 40, 60 or 80 mg/kg/day in pathological analysis.

(Key words: carbendazim, rat, teratogenicity)