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APPENDIX



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Table 1. Exposure of early 8 days mouse embryos to cadmium chloride and its effect on embryos' DNA contents.

Conc. of cadmium chloride (μM)	Total DNA content (μg)			Analysis of variance				
	N	\bar{x}	SD	Source of variation	df.	SS	MS	F
0	20	46.49	0.85					
0.5	20	42.86**	0.92	Between groups	5	7008.06	1401.612	404.068*
1.0	20	29.07**	2.59					
2.0	20	29.08**	1.63	Within groups	114	395.44	3.469	
2.8	20	29.43**	1.91					
3.0	20	27.21**	2.50	Total	119	7403.50		

Note: Embryos were explanted from pregnant mice at 8 days of gestation and cultured *in vitro* for 48 hrs in the presence of various concentration of cadmium chloride in the medium.

* More than critical values of F at $P < 0.01$

** Significantly different from controls at $P < 0.01$



Table 2. Exposure of early 8 days mouse embryos to cadmium chloride and its effect on yolk sac diameter

Conc. of cadmium chloride (μM)	Yolk sac diameter (mm.)			Analysis of variance				
	N	\bar{x}	SD	Source of variation	df.	SS	MS	F
0	20	5.14	0.04					
0.5	20	5.03	0.11	Between groups	5	3.494	0.6988	33.92*
1.0	20	4.98	0.14					
2.0	20	4.93**	0.10	Within groups	114	2.348	0.0206	
2.8	20	4.68**	0.25					
3.0	20	4.69**	0.12	Total	119	5.842		

Note: Embryos were explanted from pregnant mice at 8 days of gestation and cultured *in vitro* for 48 hrs in the presence of various concentration of cadmium chloride in the medium

* More than critical values of F at $P < 0.01$

** Significantly different from controls at $P < 0.01$

Table 3. Exposure of early 8 days mouse embryos to cadmium chloride and its effect on embryos' crown-rump length.

conc. of cadmium chloride (μM)	Crown-rump length (mm.)			Analysis of variance					
	N	\bar{x}	SD	Source of variation	df.	SS	MS	F	
0	20	4.40	0.05	Between groups	5	7.0308	1.406	188.525*	
0.5	20	4.34	0.13						
1.0	20	4.10**	0.20	Within groups	114	0.8502	0.0074		
2.0	20	4.09**	0.27						
2.8	20	3.88**	0.12						
3.0	20	3.88**	0.06						
Total					119	7.8810			

Note: Embryos were explanted from pregnant mice at 8 days of gestation and cultured *in vitro* for 48 hrs in the presence of various concentration of cadmium chloride in the medium.

* More than critical values of F at $P < 0.01$

** Significantly different from controls at $P < 0.01$

Table 4. Exposure of early 8 days mouse embryos to cadmium chloride and its effect on embryos' head length

conc. of cadmium chloride (μM)	Head length (mm.)			Analysis of variance				
	N	\bar{x}	SD	Source of variation	df.	SS	MS	F
0	20	2.22	0.02					
0.5	20	2.18	0.07	Between groups	5	1.3237	0.2647	33.94*
1.0	20	2.11**	0.20					
2.0	20	2.02**	0.11	Within groups	114	0.8895	0.0078	
2.8	20	1.96**	0.07					
3.0	20	1.95**	0.10	Total	119	2.2132		

Note: Embryos were explanted from pregnant mice at 8 days of gestation and cultured *in vitro* for 48 hrs in the presence of various concentration of cadmium chloride in the medium.

* More than critical values of F at $P < 0.01$

** Significantly different from controls at $P < 0.01$

TABLE 5 MORPHOLOGICAL RATING OF EMBRYOS DURING THE MAJOR PART OF ORGANOGENESIS

Score	0	1	2	3	4
Yolk-sac vasculature	not visible	blood islands	anastomoses of vessels	dense network; blood flow	
Allantois	free in exocoelome	connected with chorion	containing umbilical vessels		
Embryo curvature	none	ventrally convex	dorsally convex	with torsion	
Somite number	0 - 4	5 - 13	14 - 20	21 - 27	28 - 34
Heart	not visible	paired tubular hearts; no contractions	s-shaped tubular heart; contractions	3-lobe appearance	4-lobe appearance
Fore-brain	neural plate	closing	prosencephalon fused	telencephalic evaginations	hemispheres prominent
Mid-brain	neural plate	closing	mesencephalic folds closing	completely fused mesencephalon	identification of mesen- cephalon + diencephalon
Mid-brain	neural plate	closing	anterior neuropore formed	anterior neuropore closed	rhombencephalon prominent and transparent
Caudal neural tube	neural folds	elevating neural folds	partly closed from somites 2-6	posterior neuropore closed	
Maxillary process	not visible	visible	prominent		
Mandibular process	not visible	formed from 1st branchial bar			
Branchial bars	not visible	I visible	II + III visible	I + II + III visible	II overgrowing III
Optic system	not visible	rudimentary	optic bulbs	optic bulbs and stalks	lens pocket
Olfactory system	not visible	rudimentary	plate with rim	ridges	distinct ridges
Otic system	not visible	placode	cups	with dorsal recess	with endolymphatic duct
Fore limbs	not visible	rudimentary	ridges	buds	paddle shaped
Mid limbs	not visible	rudimentary	ridges	buds	paddle shaped
TOTAL SCORE					

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Table 6. Exposure of early 8 days mouse embryos to cadmium chloride and its effect on morphological score of embryos.

conc. of cadmium chloride (μM)	Morphological score			Analysis of variance				
	N	\bar{x}	SD	Source of variation	df.	SS	MS	F
0	20	43.50	0.00					
0.5	20	43.48	0.11	Between groups	5	614.117	122.82	801.226*
1.0	20	43.05**	0.28					
2.0	20	41.68**	0.37	Within groups	114	17.475	0.15329	
2.8	20	38.70**	0.57					
3.0	20	37.95**	0.61	Total	119	631.592		

Note: Embryos were explanted from pregnant mice at 8 days of gestation and cultured *in vitro* for 48 hrs in the presence of various concentration of cadmium chloride in the medium

* More than critical values of F at $P < 0.01$

** Significantly different from controls at $P < 0.01$

Table 7. Exposure of early 8 days mouse embryos to cadmium chloride and its effect on number of somites.

conc. of cadmium chloride (μM)	number of somites			Analysis of variance				
	N	\bar{x}	SD	Source of variation	df.	SS	MS	F
0	20	32.95	0.39					
0.5	20	32.50	0.51	Between groups	5	316.67	63.334	94.0092*
1.0	20	31.25**	1.48					
2.0	20	29.95**	0.83	Within groups	114	76.8	0.6737	
2.8	20	28.95**	0.69					
3.0	20	28.80	0.52	Total	119	393.47		

Note: Embryos were explanted from pregnant mice at 8 days of gestation and cultured *in vitro* for 48 hrs in the presence of various concentration of cadmium chloride in the medium.

* More than critical values of F at $P < 0.01$

** Significantly different from controls at $P < 0.01$

Table 8. Effects of *in vitro* exposure to cadmium chloride on external morphology of mouse embryos

Types of developmental anomalies. (a)	cadmium concentration (μM)					
	n = 20/group					
	0	0.5	1.0	2.0	2.8	3.0
Number of abnormal embryos	0(0)	0(0)	0(0)	1(5)	17(85)	16(80)
Number of embryos with:						
Irregular neural suture line	0(0)	0(0)	0(0)	1(5)	17(85)	16(80)
Irregular somites	0(0)	0(0)	0(0)	1(5)	17(85)	16(80)
Unfused brain folds	0(0)	0(0)	0(0)	0(0)	17(85)	16(80)
Defective flexion	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Stunted telencephalon	0(0)	0(0)	0(0)	0(0)	17(85)	16(80)

Note: Embryos were explanted from pregnant mice at 8 days of gestation and cultured *in vitro* for 48 hrs in the presence of various concentration of cadmium chloride.

(a) Figures in parenthesis give percent of abnormal embryos.

Table 9. The mortality data of pregnant mice following single i.p. administration of cadmium chloride on day 8.5 of pregnancy

Dose of cadmium chloride (mg/kg. body weight)	Number of animals studied	Number of death animals	Death rate %
4.0	17	0	0
4.2	17	5	29.41
4.5	17	7	41.17
5.0	17	9	52.94
6.0	19	15	78.95
7.0	19	18	94.73
8.0	18	18	100

Mortality was determined following single i.p. injection of cadmium chloride into pregnant mice on day 8.5 of pregnancy.



Table 10. Pregnancy and abortion rates following intraperitoneal administration of cadmium chloride to the pregnant mice.

Treatment		Number of adults		Pregnancy rate %	Abortion rate %	Mortality rate %
Days of Pregnancy	IP dose (mg/kg)	Treated	Pregnant			
7.0	Control	10	10	100	0	0
	4	14	14	100	0	0
7.5	Control	5	5	100	0	0
	4	20	20	100	0	0
8.5	Control	5	5	100	0	0
	4	20	20	100	0	0

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Table 11. Body-weight changes of female mice receiving single i.p. injection of cadmium chloride on day 7-8.5 of gestation ^{a,b}

Treatment		Number of animals	Body-weight (gm)		Body-weight gained (gm)	Body-weight gained %
Day of Pregnancy	IP dose (mg/kg)		Day of Mating	Day of autopsy		
7.0	control	10	28.52±1.80	51.56±5.57	23.00±5.19	80.65
	4.0	14	25.65±0.86	39.39±1.27	13.74±1.28**	53.56
7.5	control	5	30.86±2.44	53.78±5.82	22.92±5.00	74.27
	4.0	20	29.36±1.96	55.83±5.57	26.46±5.19	90.10
8.5	control	5	31.28±1.21	54.86±3.27	23.58±2.13	75.38
	4.0	20	26.83±1.38	49.71±3.04	22.88±2.54	85.28

^a Animals were sacrificed on day 18.5 of gestation

^b Each value represents the mean ± S.D.

** Significantly different (P < 0.01) from control

Table 12. Crown-rump lengths of fetuses.^a

Treatment		Number of Fetuses	Crown-rump lengths (cm.)	
Day of Pregnancy	IP dose (mg/kg)		Mean \pm S.D.	Mean as % of Controls
7.0-8.5	control	212	2.902 \pm 0.112	100
7.0	4.0	58	2.305 ^{**} \pm 0.078	79.43
7.5	4.0	218	2.388 ^{**} \pm 0.130	82.29
8.5	4.0	208	2.857 ^{**} \pm 0.104	98.45

Note: Pregnant mice were injected intraperitoneally with a single dose of cadmium chloride on days 7.0, 7.5 and 8.5 of gestation and sacrificed on day 18.5.

^a Crown-rump lengths were taken immediately after birth.

^{**} Significantly different from controls at $P < 0.01$ using one-way ANOVA couple with Newman-Keuls test.

Table 13. Weight of fetuses and placentae^a.

Treatment		Number of Fetuses	Weight of Fetuses (g)		Weight of Placentae (mg)	
Day of Pregnancy	IP dose (mg/kg)		Mean ± S.D.	Mean as % of controls	Mean ± S.D.	Mean as % of controls
7.0-8.5	control	212	1.368 ± 0.141	100	103.858 ± 19.777	100
7.0	4.0	58	1.181 ± ^{**} 0.115	86.33	84.862 ± ^{**} 17.317	81.71
7.5	4.0	218	1.256 ± ^{**} 0.165	91.81	85.867 ± ^{**} 24.177	82.68
8.5	4.0	208	1.383 ± 0.098	100	75.264 ± ^{**} 15.618	72.47

Note: Pregnant mice were injected intraperitoneally with a single dose of cadmium chloride on days 7.0, 7.5 and 8.5 of gestation and sacrificed on day 18.5.

^a Weight of individual fetuses and placentae were taken immediately after birth.

^{**} Significantly different from controls at $P < 0.01$ using one-way ANOVA couple with Newman-Keuls test.

VITA

Mr.Viroj Sumyai was born in Pratumthani Province and had his early education at the Don Muang School, Bangkok, Thailand. He obtained his degree in Bachelor of Science (Chemistry) from Chiangmai University in the year 1976 and Bachelor of Science in Pharmacy, Manila Central University, in the year 1980.

He is now working as a research scientist at Narcotic Control Division, Food and Drug Administration, Ministry of Public Health, Bangkok, Thailand.



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย