

Takahiro Kobayashi¹, Shinichi Kikuchi², Kazuo Komiyama³, Shinji Takahashi¹, Seishiro Hirano¹, Emiko Furuta⁴, Takahiko Yoshida⁵, Haruhisa Wago⁶

¹National Institute for Environmental Studies, ²Chiba University, ³Nihon University, ⁴Dokkyo University School of Medicine, ⁵Asahikawa Medical School, ⁶Saitama Medical School Junior College

Key words: benzo[a]pyrene, mice, quail, goldfish, pearl oyster, slug, earthworm, silkworm, phagocytosis

There is little information whether hazardous chemicals in the environments may affect the immune system in diverse species. Most studies on the effects of benzo[a]pyrene (BaP) on immune functions have been done with using experimental animals such as mice and rats not with other diverse species. Therefore, in the present study, the effect of BaP on immune functions of diverse species, such as, mice, quail, goldfish, pearl oyster, slug, earthworm, and silkworm.

Mice: Neonates were fed milk from their mothers administered with BaP intraperitoneally (10-100mg/kg day) for 7 days. On day 7, thymocytes were analyzed by flow-cytometry. Neonates from mother administered with 10 mg/kg, body weight and cellularity of thymocytes decreased. CD4-CD8- and CD4-CD8+ T-cells decreased significantly at 10 mg/kg. CD4+CD25+ T-cells decreased significantly at 100 mg/kg in low CD4+ region. These results suggested that the immune disruption occurs in mice.

Quail: Japanese quails were exposed to BaP (10, 30, 100 mg/kg) by oral administration. Mitogen activity to ConA in peripheral lymphocytes was suppressed dose dependently. Mitogen activity to LPS increased in high concentration. There was no significant differences in average SRBC-HA titer and resistance against infection challenged with Raus sarcoma virus between BaP-group and control group. These results suggested that the immune disruption in avian species occurs in cell levels and the host defence function might be changed,

Goldfish: BaP was injected into the peritoneal cavity of goldfish *Carassius auratus*, (0, 30, 100 mg/kg) and 7 d after the injection, the spleen and kidney was examined histologically/histochemically. For evaluation of phagocytic activities, carbon particles were injected 7d after BaP injection, and on the 3 d of the carbon injection, the spleen and kidney were examined by light microscopy. Degeneration caused by BaP injection on splenic and renal hemopoietic tissues was not distinct, however, in the spleen, minute decrease of phagocytic activities was histologically observed. Depressive effect on leucocytic phagocytosis in fish by BaP was suggested.

Pearl oyster: Pearl oysters were acclimated in stainless tanks with artificial seawater for 6 days. Following acclimation, five or 50 ppb BaP was solved with 0.01% DMSO to the seawater and exposed to pearl oysters for 2 weeks. Haemolymph was withdrawn from muscle and used for immunological evaluations. Agglutinin titer and haemocyte density of haemolymph, ratio of agranular cells in haemocytes, and NBT reduction activity, phagocyte activity and chemotaxis of haemocytes were evaluated. BaP exposure slightly affected phagocyte activity and chemotaxis of haemocytes but not significantly. These results suggest that BaP may induce immunosuppression on pearl oysters.

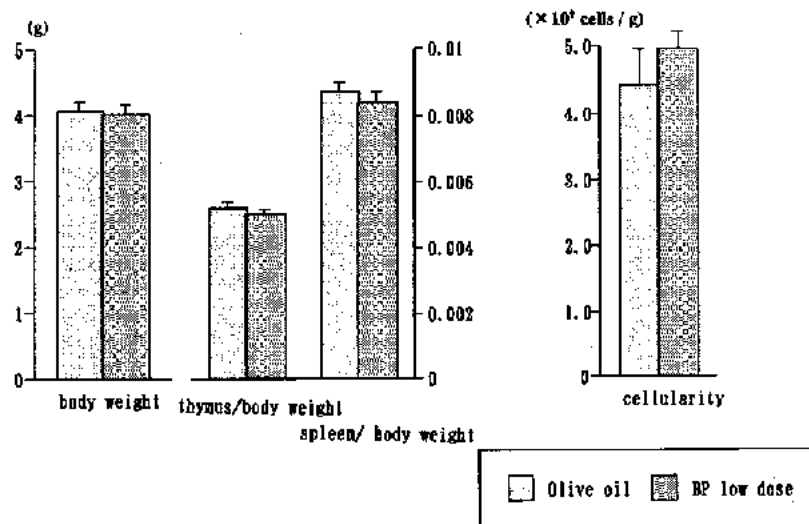
Slug: Filter papers for the slug breeding were soaked with the acetone solution of BaP at the following

concentrations (ppm) and were dried up. After five days of exposure at 5×10^3 and 10^4 ppm or 10 days at 10^1 , 10^2 and 10^3 ppm, the skin state for mucus secretion was observed and macrophages (blood cells) and some organs (the digestive, the hepatopancreas and the gonad) were removed. BaP over 10^3 ppm was decreased phagocytosis of slug's macrophages and changed drastically secreted mucus to be viscous and white, while the body mucus was usually serous and brownish. These results suggest that BaP possibly affects the internal defense mechanisms of terrestrial slugs.

Earthworm: *E.fetida* keep in the cellulose tips with 100pg to 100mg of BaP at 15°C for 1-5 days. Phagocytosis, H₂O₂ production, expression of AH receptor, estrogen receptor, cytochrome P 450, and heat shock proteins were examined. Coelomocyte was classified as large (LC) and small cell (SC) in these sizes. *E.fetida* exposed BaP for five days, clearly decreased phagocytosis and H₂O₂ activity. Immunohistochemical analysis of the *E.fetida* revealed that staining intensity was increased in P450 and HSPs, while AH receptor showed no staining. ER positive cell was not co-localized with those P450 and HSP positive cells. These results indicated that immune functions of *E.fetida* were down regulated by BaP exposure even in sub-lethal dose. The levels of immune function of the coelomocyte might be useful for the indicator of environmental chemical contaminant to soil.

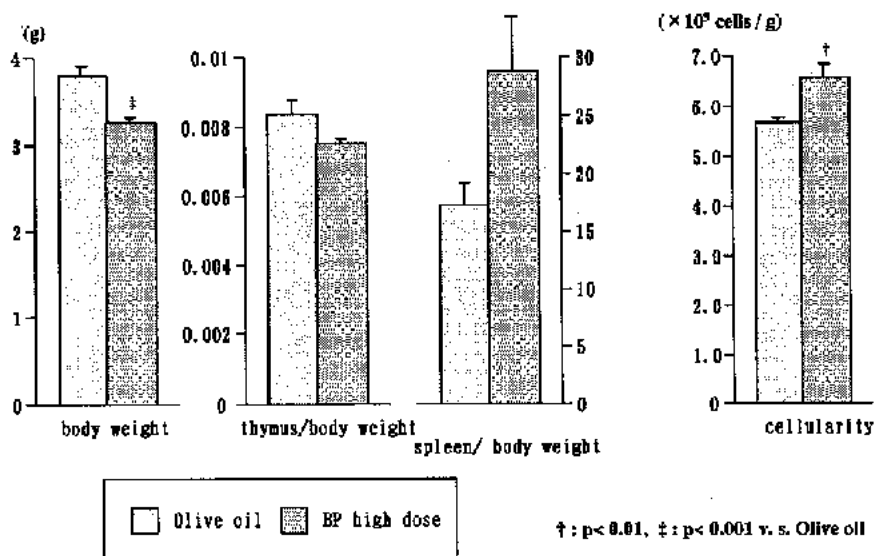
Silkworm: The consecutive application of 0.1% BP in acetone to the skin from the final 5th instar larvae once a day for 6 days resulted in a reduction of the number of total hemocytes and the proportion of granulocytes and in an increase of the proportion of plasmatocytes. In addition, erythrophagocytosis by granulocytes was also suppressed, although the formation of melanin pigment was not inhibited. Natural anti-bacterial substance and lectin molecules produced at a 2-day-old prepupal stage were investigated after an application of BP for 6 days in the 5th instar. Results with *E.coli* and human type - O blood cells showed the decrease of anti-bacterial substance and lectin levels in the hemolymph by BaP. On the other hand, the numbers of total hemocytes and immunocytes were similarly affected by only once exposure of BaP, and the in vitro treatment with BaP had an influence on the morphology of granulocytes but not of plasmatocytes leading to their round-up shape. Therefore, it is suggested that the BaP has a suppressive effect on the insect immunity, probably causing the weakness of insect health.

These results suggested that there is a possibility that BaP has the immune disrupting effects in diverse species.



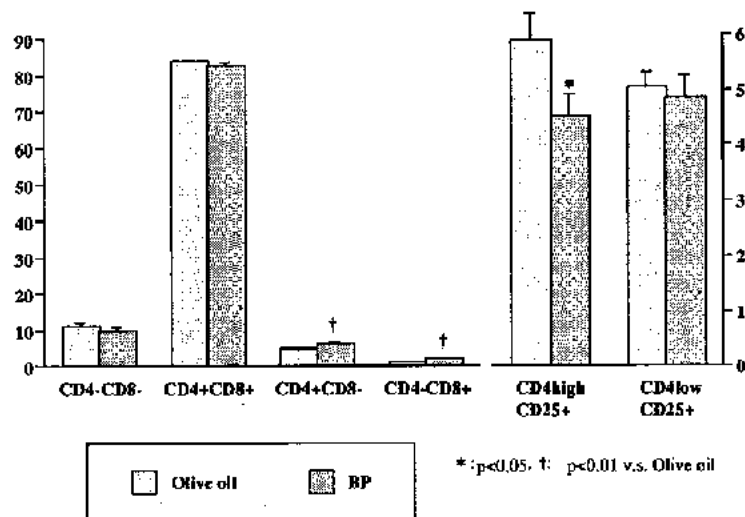
Effects of low dose BP on body weight, thymus and spleen in neonatal mice

図1. 低用量 BPがマウス新生仔の体重および免疫担当臓器に及ぼす影響



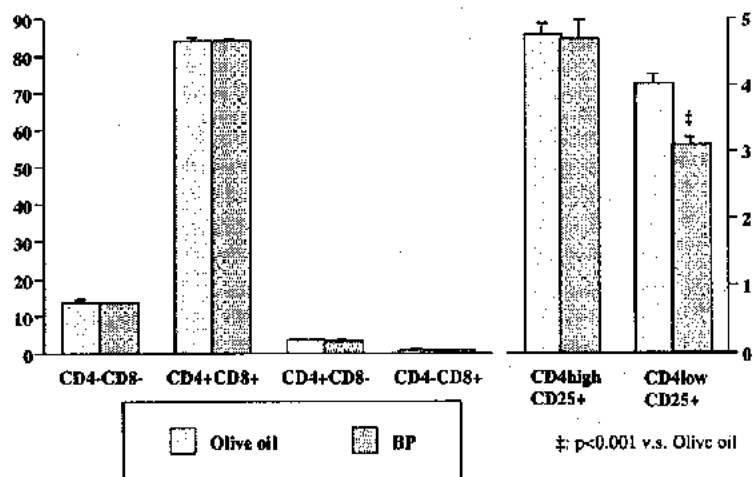
Effects of high dose BP on body weight, thymus and spleen in neonatal mice

図2. 高用量 BPがマウス新生仔の体重、免疫担当臓器におよぼす影響



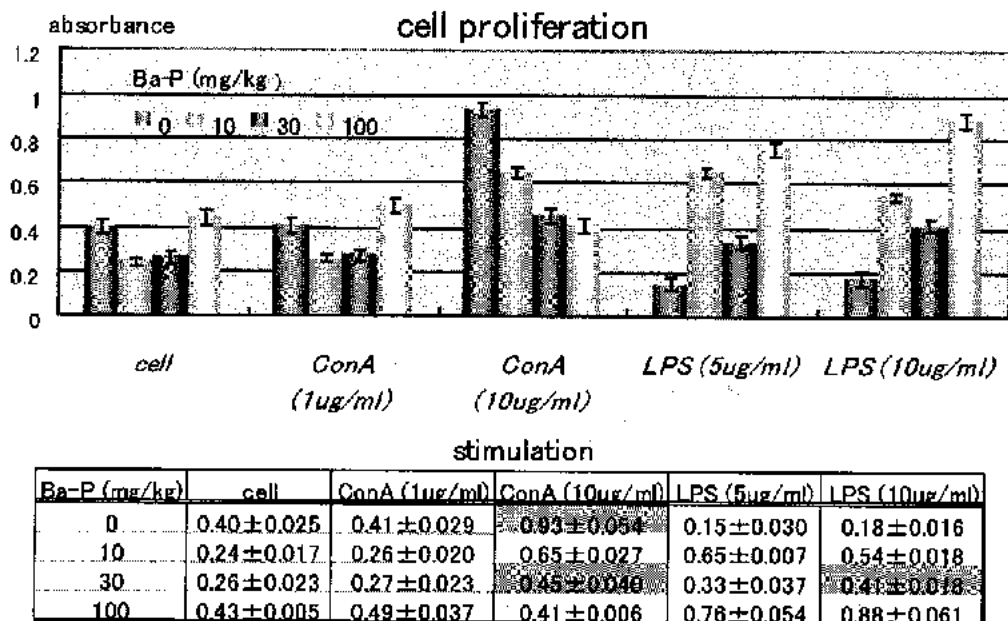
Effects of BP low dose on subsets of thymocytes in neonatal mice

図3. 低用量 BPが新生仔マウスの胸腺細胞サブセットに及ぼす影響



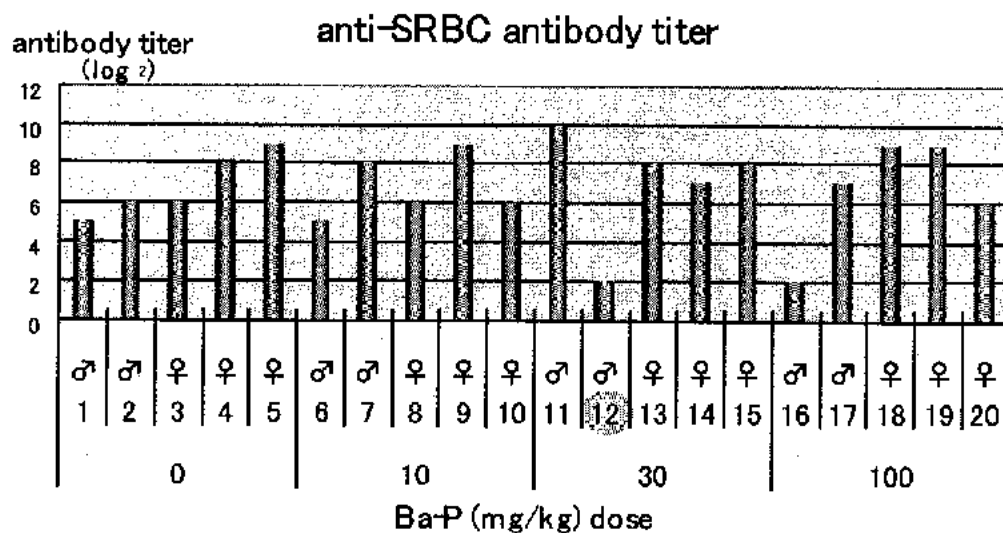
Effects of BP high dose on subsets of thymocytes in neonatal mice

図4. 高用量 BPが新生仔マウスの胸腺細胞サブセットに及ぼす影響



Change of cell proliferation to mitogens (ConA & LPS) after BP exposure

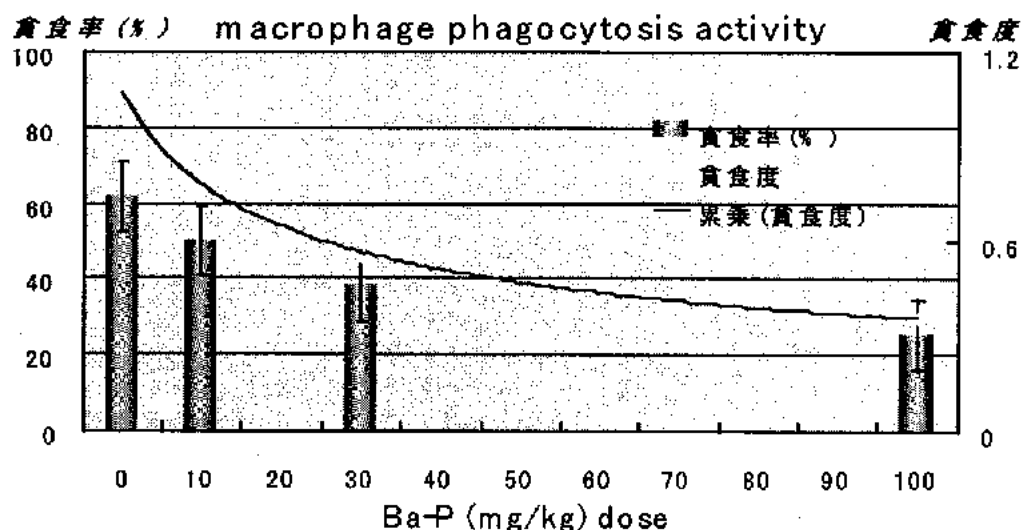
図5. Ba-P投与後のConA・LPS刺激に対するウズラリンパ球活性の変化



Ba-P (mg/kg)	0	10	30	100
antibody titer	6.8±1.64	6.8±1.64	7.0±3.00	6.6±2.88

Antibody production to SRBC after Ba-P exposure

図6. Ba-P投与後の羊赤血球に対する抗体産生能の影響



Ba-P (mg/kg)	0	10	30	100
貪食率 (%)	61.8±8.61	50.2±11.52	38.1±8.59	25.5±8.09
貪食度	1.08±0.238	0.77±0.267	0.55±0.184	0.36±0.109

Phagocytosis of *ip.* Mφ after Ba-P exposure (各 N = 5)

図7. Ba-P 投与後の腹腔内マクロファージの貪食能

RSV injection

R: right wing web L: left wing web

+: level of tumour size ±: trace

	Qual	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18 day
Ba-P+ /RSV+	G1
	G2
	G3
	G4
Ba-P+ /RSV-	N=5
Ba-P- /RSV+	G1'
	G2'
	G3'
	G4'
Ba-P- /RSV-	N=5

Comparison of RSV tumour size in wing web after BaP exposure

図8. BaP 投与後のラウス肉腫形成の比較