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Dibutyltin (DBT) Dichloride Inhibits Cytokine Productions in Murine Macrophage Cell Line, J774.1.

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Abstract:
The immune system is a target of dibutyltin (DBT) intoxication. We evaluated the effects of DBT dichloride in macrophages using the murine macrophage cell line, J774.1. Cultured J774.1 cells were exposed to DBT dichloride at 0, 0.5, 1.0, 1.5 or 2.0 μM in 24-well plates. After 18 hours, lipopolysaccharide was added to each well. The cells were incubated for an additional 6 hours or 24 hours. At the ends of the incubations, the cell viability was determined by the trypan blue exclusion method. Total RNA was extracted from the cells after an additional 6 hours of incubation. Real- time polymerase chain reaction (PCR) was used to analyze the mRNA expression for tumor necrosis factor α (TNFα), interleukin 1β (IL-1β) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (housekeeping gene) in J774.1 cells. The supernatants were sampled after an additional 24 hours of incubation. The concentrations of TNFα and IL-1β in the supernatants were determined by ELISA. The mean values of cell viabilities in the DBT-exposed groups were significantly lower than that of respective control both after the additional 6 and 24 hours of incubation. The mean relative mRNA expression of TNFα was higher than that in the control only in the 0.5 μM group. There were no significant differences in IL-1β relative mRNA expression among the groups. The mean concentrations of TNFα in the supernatant in the 1.0, 1.5 and 2.0 μM groups were significantly lower compared to that in the control. The mean concentrations of IL-1β in the supernatant in the DBT-exposed groups were also

significantly lower than that of the control. The concentrations of cytokines in the supernatants were marked low and not fully explained by the low cell viability of the DBT-exposed groups. Since the mRNA expressions of cytokines in the higher dose groups were similar to that in the control, the translation from mRNA may be inhibited by DBT.

Key words: dibutyltin, macrophages, tumor necrosis factor α, interleukin-1β, real time PCR, ELISA

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