# 学 位 論 文 内 容 の 要 旨

環境起学専攻　　博士（環境科学）　　氏 名　　劉 闖

## 学 位 論 文 題 名

A study of biological significance and toxicity of short-chain nonylphenol ethoxylates
（短鎖ノニルフェノールエトキシレートの生物的意義および毒性に関する研究）

Nonylphenol ethoxylates (NPEOs) belong to one of the largest groups of non-ionic surfactants known as alkylphenol ethoxylates. NPEOs represent majority of all alkylphenols known as alkylphenol ethoxylates. Nonylphenol (NP) production has increased exponentially, and 100 to 500 million pounds of NP are produced globally every year. NPEOs have been used in a wide variety of applications including industry as detergents, emulsifiers, wetting agents, dispersing agents, commercial and domestic uses.

After use and disposal of NPEOs, more than 60% of the products entered into the aquatic environment by the way of industrial and municipal wastewater discharges. The majority of NPEOs reach wastewater treatment systems and consequently the receiving environments. Although primary biodegradation of NPEOs is fast, it leads to more toxic and persistent metabolites: NP, nonylphenol monoethoxylate ($NP_1EO$), nonylphenol diethoxylate ($NP_2EO$), NPEOs can be biodegraded shortening of the ethoxy chain and subsequent degradation to NP under anaerobic conditions, to short-chain NPEOs ($NP_1EO$, $NP_2EO$, and $NP_3EO$) under aerobic conditions during wastewater treatment processes.

Recently, these biodegradation metabolites have been found to have endocrine disrupting properties and cause harmful effects, including feminization and carcinogenesis on various organisms. Primary NPEOs are transformed to NP mono- or di- ethoxylates ($NP_1EO$ or $NP_2EO$) under anaerobic condition, and $NP_1EC$ and $NP_2EC$ were finally degraded into NP under anaerobic conditions. In this study, in activated sludge process, absorption rates of $NP_1EO$ to $NP_3EO$ were more than 60%. It has been suggested that these compounds could be accumulated quickly in sludge at 10 min. After 24 h, although $NP_2EO$ and $NP_3EO$ contents were markedly low, $NP_1EO$ content was relative high. It was indicated that $NP_2EO$ and $NP_3EO$ were converted to $NP_1EO$ by microorganisms. Short- chain NPEOs had certain effects on COD removal.

It has been considered that NP and NPEOs are toxic to both aquatic and terrestrial organisms probably as a result of their interaction with proteins. NP and NPEOs have different structures; hence, they disrupt different biochemical processes in organisms. It has been reported that enzymes such as P-glycoprotein interact with various NPEOs, but not with NP. It was found that more than 80% of the male Medaka exposed to NP exhibited gonadal intersex. Treatment with $NP_1EO$ produced a similar outcome; however, it was reported that NP had a much stronger effect on fish and mammals compared to the short chain NPEOs. Some studies have demonstrated that NPEOs with relatively small number of EO units (s = 0-30) showed greater cytotoxicity (inhibition of cell proliferation) in human skin fibroblast cells compared to long chain NPEOs. NPEOs were found to cause direct DNA damage (DSBs, double strand breaks), but did not contribute to

reactive oxygen species (ROS). NPEOs also act as tumor initiators and promoters. In contrast, NP decreases the viability of Raji cells via ROS. These differences of the effects of NPEOs and NP may dependent on their structures. Certain $NP_{70}EO$ showed no cytotoxicity in any of the cell lines, whereas other $NP_{10}EO$ exhibited significant toxicity. With decreasing the number of EO units, cell proliferation was increasingly inhibited. Moreover, LD50 of NPEOs (s = 1.5, 9, 15, 40, and 50) in Mysidopsis bahia was 0.11, 1.41, 2.57, over 100 and over 4110 mg/L, respectively. These findings indicate that the toxicity of NPEOs increases with decreasing the number of EO units.

PC12 is a rat pheochromocytoma clonal cell line that responds to nerve growth factor by extending neurites. On the other hand, it is well known that apoptosis is induced by serum deprivation in PC12 cells. Recently, we have reported NP affects apoptosis in PC12 cells. Our results of DNA fragmentation and relative expression of apoptotic factors indicated that NP enhances apoptosis induced by serum deprivation in PC12 cells. The effects of short chain NPEOs on apoptosis have not been clarified, although it is expected that the toxicity of NPEOs will decrease with increasing the number of EO units. Investigation of effects on apoptosis is expected to contribute new insight into the mechanisms of the effect of NPEOs on the differentiation and development of an organism. The objective of our study is to investigate the role of NPEOs regulation of the changes of apoptotic status caused by serum deprivation or copper, and to clarify the mechanisms of the effects of short chain NPEOs on apoptotic conditions.

Aapoptosis has been shown to be induced by serum deprivation or copper treatment. To understand the toxicity of $NP_2EO$, we investigated the effects of $NP_2EO$ on apoptosis induced by serum deprivation and copper by using PC12 cell system. $NP_2EO$ itself showed no toxicity and recovered cell viability from apoptosis. In addition, $NP_2EO$ decreased DNA fragmentation caused by apoptosis in PC12 cells. This phenomenon was confirmed after treating apoptotic PC12 cells with $NP_2EO$, whereas the cytochrome c release into the cytosol decreased compared to that in apoptotic cells not treated with $NP_2EO$. Furthermore, Bax contents in apoptotic cells were reduced after exposure to $NP_2EO$. Thus, $NP_2EO$ has the opposite effect on apoptosis in PC12 cells compared to NP, which enhances apoptosis induced by serum deprivation. The difference in structure of the two compounds is hypothesized to be responsible for this phenomenon. These results indicated that $NP_2EO$ has capability to affect cell differentiation and development and has potentially harmful effect on organisms because of its unexpected impact on apoptosis.

In conclusion, $NP_2EO$ inhibited apoptosis induced by serum deprivation and copper. $NP_2EO$ exhibited the opposite effect on apoptosis in PC12 cells compared to NP, which might be due to the difference in the structure of the two compounds, along with different response mechanisms to apoptosis. Ultimately, further investigation is required to clarify the precise toxicity mechanism of short chain NPEOs and NP.