別紙４－１（課程博士（英文））

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Abstract（Doctor）

Title of Thesis	Neurodevelopmental Effects of Maternal Acetamiprid Exposure: From Cerebellar Changes to Behavioral Outcomes

Approx. 800 words

Neonicotinoid insecticides have gained significant attention in recent years due to their widespread use in agriculture and potential environmental impacts. Among these, acetamiprid has raised particular concerns regarding its neurodevelopmental toxicity. The structural similarity between acetamiprid and nicotine allows it to act as an agonist of nicotinic acetylcholine receptors (nAChRs), potentially disrupting normal neuronal signaling. This mechanism of action, coupled with its resistance to degradation by acetylcholinesterase, raises concerns about its long-term effects on the developing nervous system.

This thesis seeks to address the existing knowledge gap by focusing on the effects of maternal acetamiprid exposure on cerebellar neurons and glia, as well as associated behavioral outcomes in both juvenile and adult stages. This comprehensive approach aims to elucidate the potential long-term consequences of early-life exposure to this neonicotinoid insecticide. By examining both cellular changes in the cerebellum and behavioral manifestations across different developmental stages, I intend to provide a more complete picture of acetamiprid's neurotoxic effects. This investigation is crucial for understanding the full extent of the risks associated with neonicotinoid use and may inform future regulatory decisions and agricultural practices to mitigate potential harm to human and environmental health.

Chapter 1 is an introduction to the thesis, explaining the background, mainly on acetamiprid, motivations and objectives of this thesis.

In Chapter 2, I discussed the effects of maternal exposure to acetamiprid on behavioral changes in the offspring of mature animals. Animal behavior is essential to understanding the end point of any molecular, cellular, and physiological changes in response to environmental stimuli. The study of behavioral changes in mature animals following prenatal exposure to acetamiprid provides valuable insights into the long-term consequences of early-life chemical exposure. Such studies contribute to our understanding of the potential risks associated with environmental toxicants and inform regulatory decisions regarding the use of pesticides in agriculture and pest control.

In Chapter 3, I discussed the effects of prenatal exposure to acetamiprid on the offspring's behavioral test during the juvenile period. I showed that acetamiprid affects the hind leg strength of the offspring exposed to high doses

(40 mg/kg and 60 mg/kg) of acetamiprid. Acetamiprid at a lower dose (20 mg/kg) does not show any effects of acetamiprid.

In Chapter 4, I discussed the effects of prenatal exposure to acetamiprid on offspring cerebellar development. I examined the developing cerebellum of the pups on postnatal days (P) 7, 14, and 18, corresponding to the critical periods of cerebellar maturation in rodents. I showed that acetamiprid exposure at 40 mg/kg and 60 mg/kg induced abnormal neuronal alignment on P14, and neuronal cell loss on P18. Additionally, acetamiprid altered microglial behavior, with an increase in the number of CD68-positive microglia, suggesting that the exposure results in an increase in phagocytic microglia in response to neuronal abnormalities, ultimately leading to neuronal cell loss. These molecular and cellular changes may explain the behavioral outcome seen in chapters 2 and 3.

In Chapter 5, I cultured microglia from offspring maternally exposed to acetamiprid to investigate the effects of acetamiprid pesticide on changes to microglial morphology and phagocytic function in response to ATP stimulation. I showed that microglia cultured from offspring exposed to acetamiprid in utero exhibit elevated levels of interleukin-1β (IL-1β). Furthermore, IL-1β levels of the acetamiprid group were significantly increased upon ATP stimulation compared to both the control and DMSO groups. Additionally, while phagocytic properties were enhanced in the acetamiprid-exposed group, the phagocytic function of acetamiprid after ATP stimulus exhibited a decrease. From these findings, I suggest that ATP stimulus subsequent to initial microglial activation induced by acetamiprid exposure impairs microglial phagocytic function.

In Chapter 6, I proposed a new method to observe living cultured microglia and their reactivity to inflammation via the acoustic impedance mode of a scanning acoustic microscope. I observed primary cultured microglia collected from offspring exposed to acetamiprid for both acoustic interface impedance mode (C-mode) and transparent three-dimensional impedance mode (B-mode). I characterized microglia based on the results obtained from acoustic impedance, cytoskeletal information, and laser confocal imaging. Biphasic acoustic observation using B-mode and C-mode gave me information regarding the dynamic morphologies of living microglia treated with ATP. I showed that an acetamiprid exposure induced microglia response even in the neonatal period. Additionally, ATP stimulus altered the shape and thickness of microglia with a change in the bulk modulus of the cell. I showed that three-dimensional alteration with ATP stimulus could be observed only after biphasic acoustic observation using B-mode and C-mode. I showed that a scanning acoustic microscope is adequate in analyzing microglia's shape, motility, and response to inflammation.

Collectively, this investigation provides valuable insights into the effects of maternal acetamiprid exposure on offspring development through adulthood. These findings are anticipated to contribute significantly to the understanding of how maternal exposure to environmental factors, such as pesticides, influences offspring outcomes.