NEUROTOXIC EFFECTS OF PERSISTENT TOXICANTS AND ENVIRONMENTAL CHEMICAL MIXTURES

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Human exposure to persistent chemicals has been increasing, and there is a great concern about the health effects associated with long-term exposure to these chemicals. Exposure to chemical mixtures is another important issue as the number of chemicals introduced into market place are growing, and there are billions of pounds of toxic chemicals emitted into the environment each year. There is epidemiological as well as experimental evidence indicating that long-term exposure and exposure during development to environmental chemicals may cause neurotoxic effects, including learning and memory deficits. The main focus of this session is to understand the mechanism(s) involved in the neurotoxic effects of persistent toxicants and environmental chemical mixtures. The session includes both neurobehavioral effects as well as some mechanistic aspects of a variety of persistent chemicals such as polybrominated diphenyl ethers (PBDEs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated biphenyls (PCBs), and nonyl phenol. The experimental models include primary neuronal cultures, commercial human cell lines, and whole animals. Reports consist of results from rats, birds as well as zebra fish following exposure to persistent chemicals.

The first presentation is by Wiegand and colleagues of Medical Inst. of Environ. Hygiene at Heinrich-Heine University, Düesseldorf, Germany and focused on polyhalogenated hydrocarbon induced perturbation of intracellular calcium homeostasis. Results show that in different mammalian cell types, ranging from excitable cells like neurons to non-excitable cells like macrophages, persistent chemicals such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) disrupt homeostasis of Ca^{2+}. In these cell types, only PCBs with non-coplanar configuration disrupt Ca^{2+}-homeostasis, while the PCBs with coplanar configuration do not. Authors conclud that elevated intracellular free Ca^{2+} levels may have dramatic consequences for signal transduction pathways in rat astrocytes as well as human macrophages. Perturbations caused by PCBs and PBDEs seem to be similar among different cells. Authors suggest that effects of non-coplanar compounds should be considered in determining risk associated with exposure to chemicals such as PCBs.

The second talk is by Kodavanti and Derr-Yellin of USEPA in Research Triangle Park, NC, USA on the differential effects of polybrominated diphenyl ethers and polychlorinated biphenyls on [^{3}H]arachidonic (^{3}H-AA) acid release in rat neuronal cells. Authors report that a commercial PBDE mixture (DE-71) causes stimulation of ^{3}H-AA release in a concentration-dependent manner. A significant increase in the ^{3}H-AA release is seen as early as 5 min of exposure and at a concentration as low as 10 μg/ml. Further results indicate that De-71 stimulated the release of ^{3}H-AA by activating the PLA_2 pathway and this effect is similar to those caused by other organohalogen mixtures. Compared to commercial PCB mixtures, commercial PBDE mixture is three times less potent in stimulating ^{3}H-AA release in rat neuronal cells. However, PBDEs

are still in use and rapidly increasing in the environment while PCBs are banned and decreasing in the environment. Considering the structural similarity of PBDEs with PCBs and known health effects of PCBs, toxicological consequence of exposure to PBDEs should be evaluated.

The third presentation is by Fonnum and colleagues from Norwegian Defence Research Establishment in Kjeller, Norway on the effect of PCBs at the neurotransmitter receptor level in the brain. Authors report that PCBs *in vitro* did not have any effect on the receptor binding of dopamine, glutamate or muscarine ligands in cerebellar membranes. Previously, these authors reported significant effects of PCBs on the neurotransmitter uptake and storage in synaptosomal preparation. Further studies indicate that *ortho*-PCBs cause both free radical production and death of cerebellar granule cells at 8-30 μM. Both effects are ameliorated by antioxidants such as Vitamin E and NMDA receptor antagonist, MK-801 suggesting the role of oxidative stress and NMDA receptor involvement in PCB-induced toxicity in neuronal cells.

The fourth presentation is by Moon and associates from Changwon National University, Korea and KGTRI, Taejon on oxidative stress-mediated cell death in SK-N-MC cells by 2,2',5,5'-tetrachlorobiphenyl (PCB-52). Authors report that PCB-52 significantly induces a time-dependent cell death at 15 μg/ml; 26% cell death at 12 hour exposure. At 36-hour exposure, nearly 80% cell death is observed. Increased production of free radicals and lipid peroxidation is observed following exposure to PCB-52. Further studies indicate that both mannitol and superoxide dismutase display a strong scavenging activity against PCB-52 induced oxidative stress. This suggests that PCB-52 induces both oxygen free radicals and hydroxyl radicals, with more emphasis on hydroxyl radicals. PCB-52 also induces apoptotic death of SK-N-MC cells as evident from increased DNA fragmentation and poly(ADP-ribose)polymerase (PARP) proteolysis. The authors conclude that PCB 52 *in vitro* causes oxidative stress, which subsequently promoted apoptotic cell death.

The fifth presentation is by Kubota and colleagues of Oita Medical University and The University of Tokyo, Japan on angiotensin-converting enzyme (ACE) inhibitors suppress hydroxyl radical generation induced by nonylphenol in striatum. Results indicate that para-nonylphenol enhances hydroxyl radical formation and dopamine efflux induced by 1-methyl-4-phenylpyridinium ion (MPP^+). Some ACE inhibitors, such as captopril and enalaprilat, scavenge hydroxyl radicals and dopamine efflux induced by para-nonylphenol and MPP^+. The suppressive effect of ACE inhibitors on hydroxyl radical formation is based on Fenton-type reaction. The authors conclude that ACE inhibitors may protect against para-nonylphenol and MPP^+ induced hydroxyl radical formation via suppressing dopamine efflux in the rat striatum.

The sixth presentation is by Kim and associates of Kwandong University and Yeungnam University in Korea on TCDD, which inhibits cell proliferation through reduced production of reactive oxygen species (ROS) in a human neuronal cell line. Results indicate that TCDD reduces the viability of SK-N-SH cells in a dose-dependent manner and this effect may be predominantly due to suppressed cell proliferation, rather than cell death. Also, TCDD suppresses the basal generation of ROS which is not in agreement with the literature and this discrepancy may be due to different cell or tissue types. Further studies with α-naphthoflavone and 8-methoxypsoralen suggest that anti-proliferation and inhibition of ROS production induced by TCDD may be mediated through the activation of Ah-receptor (Ah-R) in SK-N-SH cells. The authors

conclude that TCDD may reduce basal generation of ROS through the activation AhR, and in turn, inhibits neuronal cell proliferation.

The seventh presentation is by Bustness and his colleagues from Norwegian Institute for Nature Research in Tromso and Norwegian Polar Institute in Oslo, Norway. Authors report the effects of long-transported organochlorines (PCB) on the behaviour of Arctic breeding glaucous gulls (*Larus hyperboreus*), two groups of birds situated in the same region, but with widely different blood levels of PCBs. Results from this field study indicate that nesting behavior, such as the proportion of time absent from the nest site when not incubating and the number of absences, is significantly related to blood concentrations of PCBs. This effect may be due to disruption of endocrine systems as PCBs are endocrine disruptors. The authors conclude that birds with higher levels of PCBs suffer some type of behavioural impairment.

The eighth presentation is by Hill and associates from University of Liverpool, UK and IBGMC, France on the effects of TCDD on early development and the neurogenesis pathway in the Zebra fish (*Danio rerio*). Results indicate that egg mortality and hatching success are not affected by TCDD exposure. However, macroscopic effects such as yolk sac oedema, pericardial oedema, and craniofacial malformations are observed following TCDD exposure. Brain necrosis is observed in some larvae at ≥80 ppt TCDD. TCDD at 400 ppt causes down regulation of transgene, GFP-neurogenin, throughout the Zebra fish brain. The authors conclude that TCDD retards development of neurological tissue at levels as low as 40 ppt and acts on the transcription of proneural genes either directly or somewhere upstream in the neurogenic cascade.