Disentangling the developmental and neurobehavioural effects of perinatal exposure to a chemical mixture found in blood of Arctic populations: differential toxicity of mixture components

Wayne Bowers[1], Jamie Nakai[0], Al Yagminas[0], Ih Chu[1], David Moir[0]

[1]Health Canada

Introduction

A wide range of environmental pollutants are known to produce neurotoxicological effects. Studies in human populations have identified relationships between exposure to persistent organic pollutants and neurological and behavioural disturbances in infants and children [1,2,3]. Exposure to ambient levels of persistent organic pollutants has also been associated with altered physical growth, immune function and thyroid hormone function in infants[2]. Studies in animals have confirmed that contaminants like polychlorinated biphenyls (PCBs), mercury, and organochlorine (OC) pesticides can disrupt a variety of neurobehavioral functions. Examples include PCB-induced deficits in motor activity [4], learning [5], memory and attention [6], neuromuscular development [7], and sensory function [8].

One of the difficulties in determining the impact of persistent chemical pollutants on human health and development is that humans are normally exposed to a wide range of pollutants and disentangling the toxicity associated with specific chemical pollutants is rather challenging. Furthermore, even though humans are rarely exposed to single contaminants, most animal toxicology studies are conducted on single chemicals and it is not clear that these provide reliable estimates of the toxicity of combined exposure to chemical pollutants [9,10]. Epidemiological studies can evaluate relationships between toxicological effects and measures of exposure to specific contaminants and provide important directions for toxicological evaluations; however, these studies cannot directly control concurrent chemical exposures and correlations between outcomes and specific components of the multi-chemical exposure and thus cannot determine if specific components are responsible for specific toxicological effects.

The current study was designed to evaluate the neurobehavioral effects of perinatal exposure to a chemical mixture that is based on relative concentrations of persistent organic pollutants found in the blood of Canadian Arctic populations and contains 14 PCB congeners, 12 organochlorine

pesticides and methyl mercury. This study compared the effects of the complete mixture with the effects of three major components of the mixture (the PCB component, the organochlorine pesticide component, and the methyl mercury component). By examining a range of neurobehavioural functions over development we also determine if specific neurobehavioural disturbances produced by the mixture can be attributed to components of the mixture and if neurobehavioural effects produced by components of the mixture are altered by concurrent exposure to other components in the mixture.

Material and Methods

Ninety-two nulliparious female Sprague-Dawley rats served as subjects. Following 14 days acclimatization, females were bred with male Sprague-Dawley rats and once a vaginal plug was detected, dams were randomly assigned to one of nine dose groups. Rats were dosed throughout gestation and lactation with corn oil vehicle, the complete mixture (0.05 or 5 mg/kg) or equivalent doses of its three major components: methyl mercury (MeHg- 0.02 or 2 mg/kg), PCBs (0.011 or 1.1 mg/kg) or organochlorine pesticides (OC - 0.019 or 1.9 mg/kg). Doses of the mixture were based on results from a previous study using this mixture. Doses were delivered daily by providing pregnant rats with small cookies laced with the assigned chemicals. Offspring were dosed in utero and via lactation and were never directly exposed to the chemicals. Litters were culled to 4 male and 4 female pups at PND 4. In addition, 2 males and 2 female pups from the culled offspring from each litter were sacrificed on PND 4, and brains, blood were collected for residue and cholinesterase analysis. Subsets of animals within each litter were sacrificed at PND 22, 30, 100 and 175 and blood, brains and livers collected for residue analysis, neurochemistry, and liver enzyme analysis. Dams were sacrificed immediately after weaning and blood, brain and liver was collected for residue analysis, cholinesterase activity and liver enzyme analysis. Measures of growth and development were monitored in all animals. Behavioural tests were conducted on subsets of offspring (typically 2 males and 2 females/litter) starting at PND 10 and with the final test at PND 160. All Behavioural testing was conducted in the dark cycle of a 12 hr light/dark cycle. Behavioural tests included: grip strength (PND 12 and 15), motor activity (PND 16 and 51), rotarod testing (PND 29), acoustic startle with prepulse inhibition (PND 21 and 49), visual discrimination learning (PND 100-130) and Morris Water Maze testing (PND 70-100). Data were analyzed with repeated measures ANOVA and raw data was $\log_{10}$ transformed where necessary. Specific contrasts were conducted using simple effects tests or post-hoc tests as appropriate.

Results and Discussion

Maternal body weight during gestation was unaltered by any treatment; however, the highest dose of the mixture and MeHg produced comparable decreases in maternal body weight during lactation. These two doses also produced comparable decreases in offspring body weight until PND 30. While the reduced body weight in offspring exposed to the highest mixture dose persisted into adulthood (PND 70), the effects of MeHg on body weight persisted into adulthood only in male offspring. No other dose significantly altered body weights. The highest mixture and MeHg doses also produced small increases in offspring mortality rates (15% increase) during lactation. While the mixture exerted no effect on post-weaning mortality, mortality rates were increased in MeHg-exposed offspring. Similar to body weight effects, post-weaning mortality was increased only in male offspring. Mortality was not affected by any other dose. Because the reductions in body weight in dams and young offspring among mixture and MeHg exposed animals were almost identical, it appears that MeHg contained in the mixture can account for the effects of the mixture

on maternal weight and early growth. The recovery of body weight reductions in female offspring and the increased mortality in male offspring exposed to MeHg provide strong evidence for sex differences in sensitivity to MeHg. The lack of sex differences in mixture-exposed offspring suggests that, at least for the persistence of body weight effects, the MeHg component of the mixture cannot account for all body weight effects. Moreover, the increased mortality in MeHg-exposed males compared to mixture-exposed males, despite equivalent doses of MeHg, suggests that the combined exposure to other chemicals modifies the lethality of MeHg.

Grip strength testing in young animals provides an index of neuromuscular development. Figure 1 shows that the highest doses of the mixture and MeHg both produced comparable reductions in grip strength at PND 12. At PND 15 both groups continued to exhibit decreased grip strength but the mixture-exposed animals exhibited significantly greater reductions at PND 15 compared to MeHg-exposed animals. The PCB and OC components of the mixture also decreased grip strength at PND 15. It does appear that the PCBs were more effective than OCs since the lowest PCB dose decreased grip strength while only the highest OC dose decreased grip strength. Because each mixture component produced decreases in grip strength that were less than the effects of the mixture, it appears that the effect of the mixture is due to the additive impact of the components.

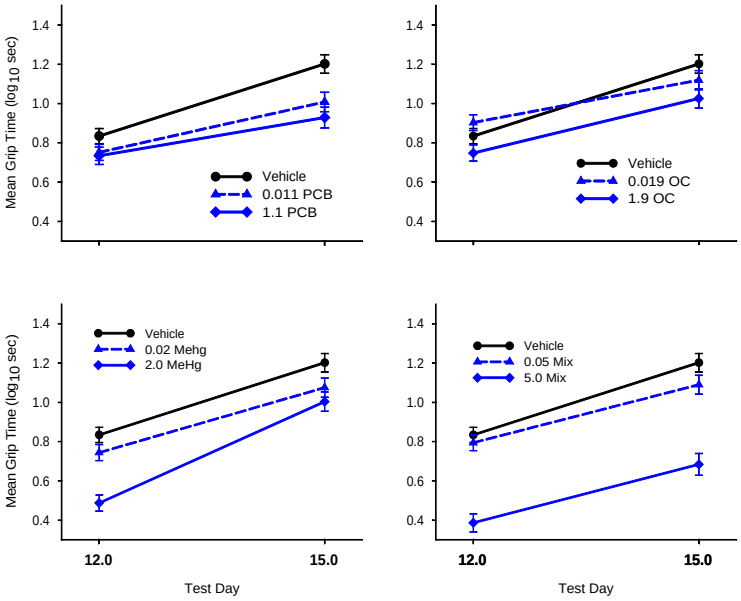
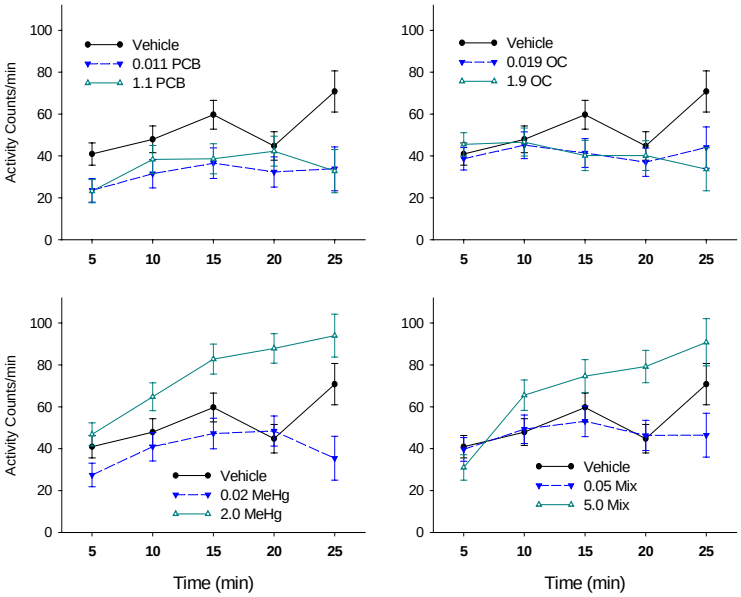
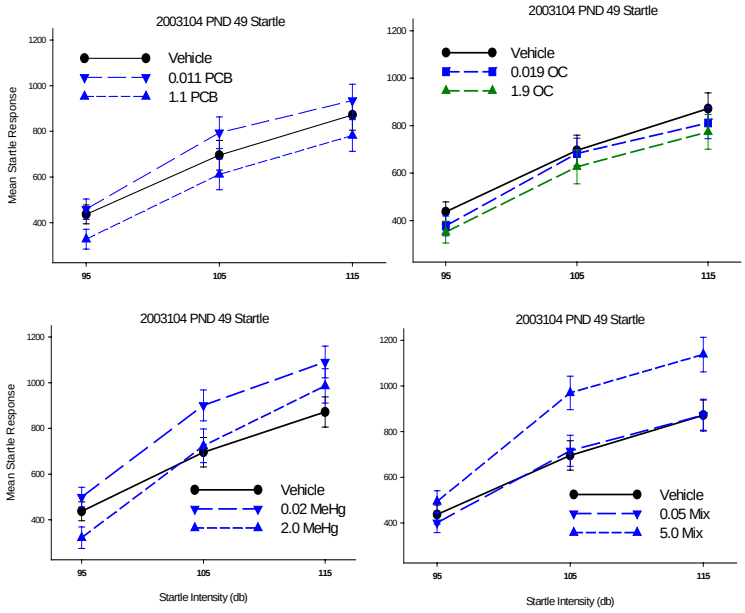


Figure 2 shows ambulatory counts in animals test at PND 16. While the highest doses of MeHg and the mixture both produced hyperactivity, the PCBs and OCs produced hypoactivity. Further, only the highest doses of the mixture and MeHg altered activity while both doses of the PCBs and OCs decreased activity. In addition to altering activity in opposite directions, the effects of the mixture and MeHg can be dissociated from the effects of the PCBs and OCs on the basis of the temporal pattern of effects. Changes in behaviour over the course of exposure to novel environments are thought to reflect a number of processes including habituation,



a form of non-associative learning, as well as emotional reactivity (fearfulness, anxiety). That the mixture-exposed animals exhibit the same temporal pattern as MeHg-exposed animals and a different temporal pattern than the PCBs and OCs suggests that the prominent effect in the mixture-exposed animals is due to MeHg. Further, the effects produced by PCBs and OCs are completely masked by the Hg component of the mixture. Changes in activity are unlikely to be related to alterations in motoric capacity since rotarod testing of motor function did not reveal any treatment-related effects. Unlike grip strength results, activity results suggest that components of the mixture do not produce additive effects and that only the MeHg component of the mixture mediates the effects of the mixture.



Sensory reactivity and sensorimotor gating (processing of multiple sensory inputs) was tested with acoustic startle with prepulse inhibition. Acoustic startle responding is a whole-body reflex response to a loud auditory stimulus. Prepulse inhibition of startle responding occurs when a brief (non-startle inducing) stimulus precedes the startle stimulus and this inhibition reflects neural processing of additional sensory stimulus. Deficits in startle responding usually reflect a disturbance in sensory processing while prepulse inhibition deficits usually reflect deficits in the integration of sensory stimuli. Figure 3 shows that the highest mixture dose increased startle responding at the two highest startle intensities used while the lowest dose had no impact. In contrast, the lowest MeHg dose increased startle responding at the two highest startle intensities while the highest dose of MeHg had no effects. The highest doses of the PCBs and OCs produced small, marginally significant reductions in startle responding. Prepulse inhibition was not affected by any dose. The increased

startle reactivity produced by the highest mixture dose does not appear to be attributable to any single component of the mixture or additive effects of the components.

One of our objectives was to determine if specific effects of the mixture could be attributed to specific components of the mixture. Body weight data suggests that the impact of the mixture on pregnant dams can be accounted for by the impact of MeHg since the reductions produced by the mixture were also produced by the same dose of MeHg administered alone. Similar results were obtained for offspring weight gain where MeHg alone produced the same reduction in weight gain as the mixture. However, in older animals the MeHg cannot fully account for the impact of the mixture and gender differences in sensitivity to MeHg became apparent in older animals while no such gender differences were evident in mixture-exposed animals. Particularly intriguing was the apparent ability of concurrent exposure to PCBs, OCs and MeHg to eliminate the post-weaning mortality in male offspring exposed to only MeHg, despite comparable effects on body weight.

The chemical mixture was effective in altering neurobehavioural function in three separate neurobehavioural tests. The contribution of major components of the mixture to the effect of the mixture depended on the specific neurobehavioural function tested. For instance, while the effect of the mixture on grip strength appears to reflect the additive impact of the separate components, this is not the case for activity where MeHg mediates the impact of the mixture even though the PCB and OC components produce opposite effects. Similarly, in the case of acoustic startle responding, no single component of the mixture appears to account for the effect of the mixture and there is no indication that additive effects of the components can account for the effect of the mixture.

These results indicate that, at least for this mixture of persistent organic pollutants, both developmental and neurobehavioural effects of the mixture cannot always be adequately characterized by the effects of specific components of the mixture. While it is the case that where the major components of the mixture alter behaviour the mixture also alters behaviour, the direction and nature of the behavioural disturbances are not necessarily consistent between the mixture and its components (e.g., PCB vs mixture on activity). For some measures, the mixture produces effects opposite to those produced by components while for other measures the impact of the mixture appear to subserved by a single component or

reflects the additive effects of the components. Overall, these results suggest that knowledge of the developmental and behavioural effects of specific components of the mixture do not necessarily provide reliable estimates of the effects of the mixture.

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