Behavioral toxicity and pharmacokinetics of PCB 77 and PCB 180 in Swiss albino mouse: A single dose inhalation toxicity risk assessment

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Research Article

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Abstract

Purpose: Polychlorinated Biphenyls pose hazards to humans as environmental and occupational workplace pollutants, despite which, though they are regulated in India, their use is not yet banned. Hence, the pharmacokinetics and behavioral effects of PCBs after inhalational exposures of Aroclor 1232 in Swiss albino mice were assessed in this study, with an objective to correlate these effects with monoamine levels in brain and extrapolate them to rodent and human risk assessments.

Methods: A whole body inhalational chamber has been utilized to expose mice to different doses of Aroclor 1232 for single six-hour durations. The plasma and brain samples were harvested at nine different time points during and after the exposures. These samples were used to estimate the corresponding concentration-time profiles of two representative congeners of PCBs (PCB 77 and PCB 180) and calculate their pharmacokinetic parameters. These variables were then correlated with mouse behavior using Irwin scale, and monoamine levels at T_{max}.

Results: A definite dose-dependence of PCB 77 and PCB 180 pharmacokinetic parameters was identified in plasma and brain. PCB 77 exhibited more accumulation in brain than plasma, whereas PCB 180 accumulated more in plasma than brain. Mood, autonomic scores, and miscellaneous sub domains were significantly different from control group scores in these domains. The changes in the mood and miscellaneous domains on the Irwin scale could be ascribed to higher brain PCB 77 $AUC_{0\text{-}t}$; whereas changes in autonomic functions could be ascribed to higher plasma PCB 180 levels. Dose-responses with monoamines followed linear (5-HT), non-monotonic (DA) and exponential (NA) relationships till 6 hours of exposure.

Conclusion: The pharmacokinetic parameters of PCB 180 and PCB 77 after acute inhalational exposures to Aroclor 1232 were obtained and this was temporally correlated with disruption of behavioral scores in dose-dependent manner. These could have a relationship with brain monoamine changes at T_{max}.

Introduction

Poly-Chlorinated Biphenyls (PCBs) are a chemical class of 209 congeners that are known to bio-accumulate, bio-magnify, bio-concentrate and persist across all the levels of the food chain. Though the production, distribution, and supply of their commercial mixtures like Aroclors and Kaneclors have been strictly regulated in most of the member countries by the United Nations Stockholm Convention[1] their replacement by best alternatives and their proper disposal are not practically feasible in many regions regulated. This is due to their favourable thermal inertness, hydraulic and dielectric nature, because of which their use in electronics, rubber and plastic industries are unavoidable; additionally, higher lipophilicity and halogenated nature make their environmental elimination practically impossible [2].

The 209 chemical congeners of PCBs can be further classified based on the number of chlorine substitutions, as low chlorine (1–4 chlorinated biphenyls) and high chlorine (5–10 chlorinated biphenyls) homolog families, each family having one to many congeners differing in the specific spatial chlorine substitution on the biphenyl nucleus. The number and the exact site of the chlorine substitution determine the nature of the congener's structure-activity relationship and its metabolism *in-vivo*, which determine its pharmacokinetics, pharmacodynamics, cumulation, and toxicological profiles. For example, ortho-substituted PCBs are known to be highly neurotoxic [3], whereas co-planar PCBs are known to possess cardiometabolic toxicities [4]. Similarly, Dioxin-like PCBs are proved to have potential to bind to Aryl-Hydrocarbon receptors in the cell nucleus and cause neuroendocrine disruption [5]. Highly chlorinated PCB congeners are known to be highly lipophilic and persist in the environment, with long biological half-lives [6]. However, the complex individual chemistry of these congeners and the variable molar composition of their restricted commercial mixtures make the predictability of the biological pharmacokinetics of PCB congeners after dosing of these mixtures complicated and difficult*in-vivo.* Aroclor 1232 is a semi-volatile commercial PCB mixture that was selected as the test substance for its ability to cause occupational air pollution indoor and outdoor. It is low chlorinated (32% Chlorine by molar mass) –hence, may not have long environmental half-lives despite *in-vivo* accumulation. It also has low vapor pressure such that it can also leach soil from air and be carried out to water bodies [2]. PCB 77 is a Dioxin like tetra-chloro-biphenyl (Tetra CB) that is also semi-volatile but is highly potent in causing neuroendocrine toxicities after binding to Aryl Hydrocarbon receptors [4]. PCB 180 is a highly lipophilic hepta-CB congener that is known to persist in biological systems and cause cardio-metabolic toxicities [6]. PCB 180 has higher octanol-water partition coefficient than PCB 77 [2].

As a blind screening tool, the Irwin neurobehavioral scale is a validated scale to be used to assess the cross sectional behavioral, autonomic, neurological, and physiological status of the laboratory mouse, both in normal and treated animals [7]. Additionally, it can be used to delineate the pharmacological effects of diverse groups of drugs acting on the nervous system. Using existing HPLC-FLD-based approaches for measurement of monoamines, the study evaluated the behavioral changes with pharmacokinetic time-points in relation to monoamine concentration changes.

This study aims to predict the pharmacokinetic variables of PCB 77 and PCB 180 after inhalation dosing of a PCB commercial mixture, viz., Aroclor 1232, in mice housed in a whole-body inhalation chamber, in an acute exposure protocol. The study then enables to study further the relationships of the PCB 77 and PCB 180 levels in brain and plasma with behavior scores assessed by Irwin scale. After dosing this mixture in mice via inhalation chamber, the behavioral effects of PCB 77 and PCB 180 have been determined using estimations of their plasma and brain concentration-ratios at geometrically progressive dose increments.

Materials And Methods

Chemicals

Aroclor 1232 for inhalation exposure was purchased from Sigma-Aldrich Inc., India. Reference standards PCB 77, PCB 180, PCB 101, 5-Hydroxytryptamine (5-HT), Dopamine (DA), Nor-adrenaline (NA) and Paracetamol were obtained from Sigma-Aldrich Inc., India. PCB 101 was used as an Internal Standard (IS). Solvents including methanol, tert-Butyl Methyl Ether (TBME) and acetonitrile (ACN) were purchased from Sigma-Aldrich Inc., India. High-purity water for chromatographic analyses was purchased from Merck-Millipore Inc., India.

Animals

Institutional Animal Ethics Committee (IAEC) approval was obtained prior to the conduct of the study for utilization of Swiss albino mice, which has been recognized by the Indian Regulatory authority, the Committee for the Purpose of Control and Supervision of Experiments on Animals [8]. All mice were femalesabout 16-32 gm at initiation and were housed in pairs per cage. Before study initiation, all the animals were acclimatized, and allocated to the three dose levels randomly. Except during the exposure period, they were provided with water and feed *ad-libitum* and housed under standard circadian rhythm and housing conditions as per OECD guidance for using humane endpoints in toxicity studies as applied to inhalation toxicity [9].

Methods:

Inhalation Chamber and the Exposure protocol

Components of a whole-body inhalation chamber which was used for exposure of test chemical in mice consisted of five units – the inlet, the animal exposure-control unit, the outlet, the connecting lines, and the external indoor environment [10]. Exposures to Aroclor 1232 consisted of three test doses in geometric progression (2.0, 4.0 and 16.0 $mg.m^{-3}$). Each dose of the test substance was given to a group of 36 mice in the inhalation chamber as per OECD guidelines for sub-acute inhalation toxicity [9]. A cohort of four animals per time point was procured out from the chamber for each time point (pre-dose, 2.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0 hours) for a total of nine time-points per dose level. The internal chamber environmental variables like oxygen content, relative humidity, temperature, pressure, and flow rate of air through the inlet along with the Aroclor 1232 solution were monitored and controlled for ensuring proper animal welfare.

Irwin Behavior assessment

It required about three to eight minutes of testing per mouse for its behavioral rating scores using Irwin scale. The scale consists of about forty-seven different functions and processes, divided into eleven domains, each affecting the central, peripheral, and somatic, and the autonomic nervous system, with effects related to cardiovascular and respiratory systems. Certain of these functions or sub domains can be tested by observation, whereas others can be assessed in the mouse after gentle maneuvers. Each sub domain has a minimum score, a normative score, and a maximum score, ranging from 0-4.

The initial undisturbed observation consisted of assessment of body position, locomotor activity, exophthalmos, respiration, tremors, twitches, convulsions, exploratory activity, hostility, and social conglomeration, as the mice remained inside the chamber. The animal was scored on a scale of 0-4 for each of these sub domain functions. After completion of this initial observation, the mouse was transferred from the chamber into the cage and then gently manipulated towards the floor of the chamber for response grading to assess the transfer arousal, spatial locomotion, palpebral closure, startle response, piloerection, tremor grading, gait and limb rotation. Following these scoring steps, the animal is lifted vertically by its mid tail above a wired mesh grid, and lowered slowly for assessment of visual placing response, i.e., extension of all limbs before contact. Grip strength and positional struggle behavior were then assessed. Body tone, gross signs of hypothermia, pinna and corneal reflexes, toe-pinch response were then graded. After this, the mouse was transferred to the chamber and graded for alley progression, withdrawal responses, touch-escape response, and positional passivity. Finally, autonomic signs like diarrhea, skin / fur colour, limb tone, abdominal tone, pupillary reflex, lacrimation, salivation, pupillary size, and provoked biting response (for aggression) were observed followed by tail-pinch response, righting reflex, provoked freezing, grasp irritability, vocalization and defecation and urination assessments [7].

Sample collection

Plasma collection and processing

Before euthanizing PCB-exposed mice at each time point for dissection and homogenization of brain samples by high dose Ketamine (55 mg/kg I.P.) (Leary *et al*., 2013), their blood samples were collected by a terminal cardiac puncture (maximum 0.2 ml) in tubes premixed with 0.012 M buffered calcium disodium EDTA in the ratio of 3:1 of anticoagulant solution: blood, and these were then centrifuged at 3000 rpm for 8 minutes at 4°C immediately. The centrifuged sample volumes were then separated for their plasma component, and the plasma was stored at -80 ± 10°C, until analysis.

Brain collection and processing

The euthanized mice brains were dissected out as whole; homogenized for storage in 1% of ice-cold Phosphate Buffered Saline (PBS) solution in -80 ± 10ºC until analysis. Blank brain samples were collected at 0 hours pre-dosing for calibration purposes. Each brain sample was divided into two equal portions for estimation of PCB levels and monoamine levels, respectively.

Instrumentation and Chromatography conditions for PCB estimation

The Ultra-High-Performance Liquid Chromatography (UPLC) instrument consisted of a Waters Acquity H class UPLC system equipped with a quaternary pump and 96-vial autosampler coupled with a diode array UV detector (Waters, Milford, MA, USA). The chromatographic separation was performed on an Acquity UPLC BEH C18 column from Waters (2.1 mm × 100mm; 1.7µm). The column temperature was set at 35ºC and the autosampler was kept at 15ºC.

The mobile phase composed of a mixture of acetonitrile (85%, v/v) and water (15%, v/v) at a flow rate of 0.3ml/min. Before analysis, the mobile phase was filtered through a 0.22µm membrane filter and degassed by ultra-sonication. A 5µl injection of each sample was loaded on to the system and total analysis time was 6 min. The Diode Array Detector (DAD) was set at 215 nm. The sampling needle was washed with 300µl of strong wash (ACN/water, 65/35) to reduce carry-over and 300µl of weak wash (ACN/water, 30/70). Data acquisition was done using Empower 3 software version 1.0 (Waters, USA) [11].

Preparation of stock and working standard solution for PCB estimation

PCB 77 and PCB 180 stock solutions of 1mg/mL were prepared by dissolving a suitable amount of single standard in methanol. Mixture of stock solutions PCB 77 and PCB 180 (100 µg/mL) were prepared in methanol. The IS stock solution of 200 µg/mL was prepared in methanol. The stock solution of PCB 77, PCB 180 and IS were stable at 4°C for one month. Further dilution was made in methanol-water (50:50, v/v) to produce working stock solution for the calibration standards and quality control (QC) standards. The IS working solution (5 µg/ml) was prepared in methanol-water (50:50 v/v). Calibration curve samples were prepared in drug-free mouse plasma with the appropriate mixture of working solution of PCB 77 and PCB 180 on the day of analysis. All the samples were stored together at -80 ± 10°C until analysis.

Sample Preparation for Plasma quantification of PCB congeners

Sample preparation was carried out by the liquid-liquid extraction procedure. To a 100 µL of aliquot of plasma, 10 µL of IS working solution was mixed for 30s on a spinix vortex shaker (Tarsons, India). To this, 3.0 mL of tert-butyl-methyl-ether was added, and this was vortex mixed for 5 min. Then this was centrifuged at 10,000 rpm for 5 min at 4ºC on an Eppendorf 5810R centrifuge (Eppendorf AG, Hamburg, Germany). The clear supernatant organic layer (2.5 mL) was transferred into 5 mL polypropylene tubes and evaporated to dryness at 35ºC using nitrogen evaporator (Turbovap®, Biotage, USA). The residue was reconstituted in 100 µL of the mobile phase, vortex mixed for 1.0 min and centrifuged at 10,000 rpm for 5 min. Finally, 90 µL of the clear supernatant were transferred into glass micro-vials and 5µL were injected onto the UPLC system for analysis.

Sample Preparation for Brain quantification of PCB congeners and monoamines

Sample preparation was carried out by the liquid-liquid extraction procedure. To a 500 µL of aliquot of brain homogenate, 50 µL of IS working solution was mixed for 30s on a spinix vortex shaker (Tarsons, India). To this, 3.0 mL of tert-butyl-methyl-ether was added, and this was vortex mixed for 5 min. Then this was centrifuged at 10,000 rpm for 5 min at 4ºC on an Eppendorf 5810R centrifuge (Eppendorf AG, Hamburg, Germany). The clear supernatant organic layer (2.5 mL) was transferred into 5 mL polypropylene tubes and evaporated to dryness at 35ºC using nitrogen evaporator (Turbovap®, Biotage, USA). The residue was reconstituted in 500 µL of the mobile phase, vortex mixed for 1.0 min and centrifuged at 10,000 rpm for 5 min. Finally, 200 µL of the clear supernatant were transferred into glass micro-vials and 5µL volumes were injected onto the UPLC system for analysis [11].

Parent monoamine analysis measurements

5-HT, DA and NA stock solutions of 1mg/mL were prepared by dissolving a suitable amount of single standard in acetonitrile. Mixture of stock solutions 5-HT, DA and NA (100 µg/mL) were prepared in acetonitrile. The High-Performance Liquid Chromatography (HPLC) instrument consisted of a Waters 515 HPLC system equipped with an isocratic pump and coupled with a fluorescence detector (Waters, Milford, MA, USA). The chromatographic separation was performed on a Gemini-C18 column from Phenomenex (4.3 mm × 50mm; 3µm).Buffer was prepared using 70 mM potassium dihydrogen phosphate, 0.1 mM dipotassium EDTA salt, 1.1 mM heptane sulfonic acid and 0.4 % Trifluoracetic acid added to 1000 mL water and adjusted to a pH of 3.14 using saturated citric acid solution. The mobile phase composed of a mixture of methanol (10%, v/v) and buffer (90%, v/v) at a flow rate of 0.5ml/min. Paracetamol used as internal standard (IS). The mobile phase was filtered through a 0.22µm membrane filter and degassed by ultra-sonication. The fluorescence detector was set excitation of 285 nm and emission at 345 nm respectively. Data acquisition was done using Empower 2 software version 1.0 (Waters, USA) [12].

Statistical analysis

Data were represented as mean ± standard deviation. Win-Non-Lin software 5.1 was used to calculate the pharmacokinetic parameters by non-compartmental model (Pharsight Corporation, CA, and USA). Pearson correlation was used to assess the relationships between behavioral scores in each sub-domain and the time-points for each dose level. One-way Repeated Measures ANOVA was used to evaluate the significance of each correlation coefficient obtained pair wise in Pearson correlation test. Hill model was used to model dose-response-effects versus brain:plasma ratios for each congener estimated using Inhibitory-Dose (ID-50) modeling. Pearson Correlation, one-way Repeated Measures Analysis of Variance (RM-ANOVA) and Dose response modeling (Hill model, and monoamine analysis) were performed using GraphPad Prism version 8.4.2 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

Results

The pharmacokinetics of PCB 77 and PCB 180 in plasma and brain were similar with small but notable differences in a dose-dependent manner (Supplemental files 1A and 1B). These parameters could be correlated to central behavioral effects based on brain pharmacokinetics, and autonomic nervous system effects based on plasma pharmacokinetics, in dose-dependent and time-dependent manner (Supplemental files 3A and 3B). Generally, brain accumulation of PCB 77 was more significantly responsible for behavioral disruption than PCB 180 accumulation in plasma – based on brain-plasma ratios of both these congeners (Supplemental files 2A and 2B).

Pharmacokinetics profile of PCB 77 at 2.0, 4.0 and 16.0 mg.m^{-3} in mice plasma:

The mean maximum concentration attained, C_{max} of PCB 77 in plasma was 88.32 ± 8.28 ng.ml^{-1} for the 2 mg.m^{-3} group. The time taken to achieve this value of C_{max} in the plasma of low dose exposure group was 3.50 ± 1.00 hr. The AUC_{0-t} for PCB 77 in plasma was 1321.19 ± 55.72 ng.hr.ml^{-1}. The Area Under the plasma Concentration-time (infinity) Curve, $AUC_{0-\infty}$ in plasma was 1626.02 ± 64.13 ng.hr.ml^{-1} for PCB 77 after dosing 2 mg.m^{-3} of Aroclor 1232. The elimination rate constant, K_{el} in plasma for PCB 77 after dosing mice with 2 mg.m^{-3} of Aroclor 1232 by inhalation route was 0.03 hr^{-1}. Plasma half-life of PCB 77 in this exposure group was 20.17 ± 1.38 hr. The Mean plasma Residence Times (MRT) for PCB 77 in this dose level was 16.51 ± 0.42 hr. The plasma apparent volume of distribution (Vd) and clearance (CL) for PCB 77 after dosing Aroclor 1232 in this group of mice were 35858.25 ± 3073.55 and, 1231.46 ± 49.43 ml.hr^{-1}.m^{-2}, respectively.

In the 4 mg.m^{-3} exposure group, the C_{max} and T_{max} of PCB 77 in plasma were 245.62 ± 39.58 ng.ml^{-1} and 4.00 hr, respectively. The AUC_{0-t} and $AUC_{0-\infty}$ of PCB 77 in this intermediate dosing group were 2749.28 ± 392.00 and 2957.28 ± 413.20 ng.hr.ml^{-1} in plasma. The K_{el} values were 0.06 hr^{-1} in plasma of the mice treated with 4 mg.m^{-3} group, while the half-life was 12.72 ± 0.36 hr in this group. The MRT in the intermediate dose exposure groups was 14.31 ± 0.49 hr in the plasma. Finally, the Vd and CL in plasma of this exposure level were 25209.01 ± 3835.06 ml.m^{-2} and 1375.07 ± 215.15 ml.hr^{-1}.m^{-2}.

In the 16 mg.m^{-3} group, the C_{max} of PCB 77 was 727.40 ± 42.52 ng.ml^{-1} in plasma and T_{max} was 4.00 hr. The mean AUC_{0-t} and $AUC_{0-\infty}$ in the plasma of this high dose group were 7565.21 ± 148.44 and 8013.39 ± 263.11 ng.hr.ml^{-1}. In the high dose group, the $K_{el}$and $t_{-1/2}$ in plasma were 0.06 hr^{-1}, and 11.17 ± 1.54 hr, respectively. The MRT of PCB 77 in plasma of the mice treated with high dose of Aroclor 1232was 14.29 ± 0.24 hr. The high dose group Vd and CL in plasma was 32099.78 ± 3593.04 ml.m^{-2} and 1998.28 ± 65.96 ml.hr^{-1}.m^{-2} in plasma.

The mean plasma concentration-time profile of PCB 77 in mouse plasma after inhalation of PCB mixture at three different dose levels are presented in Figure 1, inset A, and Table 1.

Pharmacokinetics profile of PCB 77 at 2.0, 4.0 and 16.0 mg.m^{-3} in mice brain:

The mean maximum concentration attained; C_{max} of PCB 77 was 69.43 ± 10.54 ng.ml^{-1} in brains of mice treated with 2 mg.m^{-3} of Aroclor 1232 via inhalation route. The T_{max} of PCB 77 in brain tissues of mice exposed to this dose was 3.50 ± 1.00 hr. The AUC_{0-t} for PCB 77 in brain was 1254.97 ± 57.51 ng.hr.ml^{-1}. The $AUC_{0-\infty}$ was 1469.62 ± 161.80 ng.hr.ml^{-1} for PCB 77. The elimination rate constant, K_{el} in brain for PCB 77 was 0.05 hr^{-1}. Brain half-life of PCB 77 in this exposure group was 16.20 ± 4.36 hr. The Mean brain Residence Times (MRT) for PCB 77 in this dose level was 17.07 ± 1.93 hr. The values for the apparent volume of distribution and tissue clearance of PCB 77 in brain were 31481.64 ± 5927.66 ml.m^{-2} respectively, and 1373.32 ± 150.80 ml.hr^{-1}.m^{-2}, respectively.

In the 4 mg.m^{-3} exposure group, the C_{max} and T_{max} of PCB 77 in brain were 175.13 ± 6.42 ng.ml^{-1}, and 4.00 hr, respectively. The AUC_{0-t} and $AUC_{0-\infty}$ of PCB 77 in this group were 2581.12 ± 136.46 and 2366.40 ± 142.19 ng.hr.ml^{-1}, respectively. The K_{el} values were 0.05 hr^{-1} in brain of the mice treated with 4 mg.m^{-3} group, while the half-lifeof PCB 77 in brain was 14.20 ± 0.58 hr in brain. The MRT in the intermediate dose exposure group was 15.05 ± 0.32 hr in brain, while the Vd and CL in brain of this exposure group were 31856.13 ± 3012. 73 ml.m^{-2} and 1553.12 ± 85.97 ml.hr^{-1}.m^{-2}.

In the 16 mg.m^{-3} group, the C_{max} of PCB 77 was 526.21 ± 28.63 ng.ml^{-1} in brain and T_{max} was 4.00 hr. The mean AUC_{0-t} and $AUC_{0-\infty}$ in the brain of this group were 6798.93 ± 340.47 ng.hr.ml^{-1}and 7100.56 ± 413.77ng.hr.ml^{-1}. The K_{el} and $t_{-1/2}$ in brain were 0.07 hr^{-1}, and 10.82 ± 1.29 hr, respectively. The MRT was 14.39 ± 0.30 hr. The Vd and CL in brain were 35070.00 ± 2110.32 ml.m^{-2} and 2258.90 ± 127.06 ml.hr^{-1}.m^{-2}.

The mean brain concentration-time profile of PCB 77 in mouse brain after inhalation of PCB mixture at three different dose levels are presented in Figure 1, inset B, and Table 1.

Pharmacokinetics profile of PCB 180 at 2.0, 4.0 and 16.0 mg.m^{-3} in mice plasma:

The mean C_{max} of PCB 180 in plasma after 2 mg.m^{-3} inhalation of Aroclor 1232 was 66.49 ± 5.22 ng.ml^{-1}. The time taken to reach this value of C_{max} in plasma was 2.00 hr. The AUC_{0-t} and $AUC_{0-\infty}$ attained by PCB 180 in plasma in this dose level were 763.27 ± 38.80 and 1226.52 ± 136.72 ng.hr.ml^{-1}, respectively. The Elimination rate constant of PCB 180 for its elimination from plasma at this dose level was 0.02 hr^{-1}. Its half-life in plasma was 28.95 ± 3.39 hr. Its MRT in plasma was 13.11 ± 0.96 hr at this dose level. The plasma Vd and CL of PCB 180 after administration of 2 mg.m^{-3} of Aroclor 1232 for 6 hr were 68083.41 ± 1151.95 ml.m^{-2}, and 1646.03 ± 184.36 ml.hr^{-1}.m^{-2}.

The mean C_{max} of PCB 180 in plasma in the 4 mg.m^{-3} group was 370.21 ± 42.59 ng.ml^{-1}, and the time taken to reach this concentration in plasma (T_{max}) was 2.00 hr. The AUC_{0-t} and $AUC_{0-\infty}$ were 4369.75 ± 730.55 and 5425.39 ± 408.95 ng.hr.ml^{-1}. This congener's elimination rate constant and half-life were 0.04 ± 0.01 hr^{-1} and 20.04 ± 6.43 hr, respectively. MRT in plasma for this group was 15.43 ± 0.85 hr; with a Vd and plasma CL of 21729.82 ± 8734.97 ml.m^{-2} and 740.51 ± 57.37 ml.hr^{-1}.m^{-2}, both somewhat less compared to the lower dose level.

In the 16 mg.m^{-3} exposure, the time taken to reach a plasma C_{max} of 829.57 ± 31.40 ng.ml^{-1} was 6.00 hr. The corresponding AUC_{0-t} and $AUC_{0-\infty}$ for the plasma-concentration-time relationship in this group were 13192.39 ± 498.68 and 15636.03 ± 1053.10 ng.hr.ml^{-1}. The K_{el} and $t_{-1/2}$ in this group were 0.04 ±

0.01 hr^{-1} and 18.21 ± 1.94 hr, respectively, and the MRT was 16.24 ± 0.32 hr. The Vd and CL of PCB 180 from plasma at this dosing level were 26825.47 ± 1287.33 $ml.m^{-2}$ and 1026.94 ± 72.53 $ml.hr^{-1}.m^{-2}$, respectively.

The mean plasma concentration-time profile of PCB 180 in mouse plasma after inhalation of PCB mixture at three different dose levels are presented in Figure 2, inset A, and Table 2.

Pharmacokinetics profile of PCB 180 at 2.0, 4.0 and 16.0 mg/m^3 in mice brain:

The peak concentration of PCB 180 attained in the brain in the 2, 4 and 16 $mg.m^{-3}$ groups were42.04 ± 4.83, 135.49 ± 4.95 and 429.35 ± 14.37 $ng.ml^{-1}$, respectively, showing a geometrically progressive trend with dose. The T_{max} of this congener in brain were 2.0 ± 0.0, 2.0 ± 0.0, and 6.0 ± 0.0 hr in the three dose levels, respectively. The AUC_{0-t} in brain were 631.12 ± 46.60, 2449.37 ± 186.79, and 8332.99 123.54, $ng.hr.ml^{-1}$ respectively with dose increments; the $AUC_{0-\infty}$ for PCB 180 across the three dose levels were 915.48 ± 106.63, 3896.91 ± 367.99, and 10984.01 ± 590.37 $ng.hr.ml^{-1}$, respectively. The K_{el} in brain were 0.03, 0.02 and 0.03 hr^{-1} across the three graded doses of Aroclor 1232. The half-lives of PCB 180 elimination from brain were 22.17 ± 3.24, 31.43 ± 3.10, and 22.73 ± 3.57 hr, while the MRT were 13.88 ± 0.21, 19.45 ± 0.64, and 18.72 ± 0.21 hr correspondingly, in the brain. The mean apparent Vd of PCB 180 in the mouse brains in the 2, 4 and 16 $mg.m^{-3}$ doses were 69838.36 ± 5306.26, 46934.13 ± 6867.21 and, 47593.68 ± 4968.74 $ml.m^{-2}$ and its clearance were 2207.04 ± 256.46, 1033.24 ± 96.14 and 1459.71 ± 75.49 $ml.hr^{-1}.m^{-2}$.

The mean brain concentration-time profile of PCB 180 in mouse brain after inhalation of PCB mixture at three different dose levels are presented in Figure 2, inset B, and Table 2 (Supplemental files 1A and 1B).

Dose dependence of plasma PCB 180 and PCB 77:

Aroclor 1232 was dosed to Swiss albino mice at three dose levels – 2.0 (low dose), 4.0 (intermediate dose) and 16.0 (high dose) $mg.m^{-3}$. PCB 77 C_{max} in plasma was eight times higher in high dose compared to low dose level. However, this was only three times higher in intermediate dose group compared to 2.0 mg/m^3 dose level and three times higher for high dose compared to 4.0 mg/m^3. In plasma, PCB 180 C_{max} was six times and twelve times higher for intermediate and high dose groups compared to 2.0 mg/m^3 dose levels. The high dose level C_{max} for plasma PCB 180 was twice than intermediate dose level.

Dose-dependent translation of brain to plasma PCB 77, 180 ratios:

PCB 77 brain to plasma ratios were similar for intermediate and high dose level, which reached about 1.1 times for lower dose level. The ratios for PCB 180 were three times in the intermediate dose level compared to high dose; the ratio was 5 times higher in low dose compared to the high dose level, and 1.7 times high in intermediate dose compared to low dose (Figure 3, Table 3, Supplemental files 2A and 2B).

Effect of brain to plasma ratio of PCB 77 and PCB 180 in Swiss albino mice:

Hill model was used to quantify the Inhibitory Dose, ID-50 values for PCB 180 and PCB 77 to depress behavioral scores at the same level of brain to plasma ratios (Figure 3, and, 4). PCB 180 ID-50 was 7.645 $mg.m^{-3,}$ and this was higher than PCB 77 ID-50 values (7.382 $mg.m^{-3}$). The slopes of lines were 13.89 and 12.42, and *Y*-Intercepts for behavioral disruption were 38.23 and 37.16 respectively for PCB 77 and PCB 180.

Effects of Aroclor 1232 on behavior:

There was a significant difference in total scores at 3 and 4 hours for all three doses. Significant difference in mean scores was also observed at 5 hours of single-6-hour exposures for 2 and 4 $mg.m^{-3}$ doses ($p< 0.05$), when compared to control group. The sub scores that contributed to greater than 50% deviation from the normative values, as assessed by Mann Whitney U test were distributed across three main domains – mood, autonomic activity, and miscellaneous functions, for all dose levels. The C_{max} of 2, 4, and 16 $mg.m^{-3}$ dose groups were achieved at 3.5 ± 1.00, 4.0 ± 0 and 4.0 ± 0 hr for PCB 77 in plasma and brain. The T_{max} of PCB 180 plasma and brain at 2, 4, and 16 $mg.m^{-3}$ dose levels were 2.0 ± 0, 2.0 ± 0 and 6.0 ± 0 hr. A more detailed analysis of subdomain score across doses revealed that grooming and restlessness (mood), urination, heart and respiratory rates, skin colour and palpebral opening (autonomic), and exploratory and group clustering behaviors (miscellaneous domain) were contributing to the statistical significance in the behavioral changes compared to control group (Supplemental files 3A and 3B).

Brain monoamine concentrations at $T_{max}$of PCB congeners after exposures to different doses of Aroclor 1232:

The brain concentrations of Noradrenaline at 4 hr (C_{max} of PCB 77 in brain) were 3.8, 1.7 and 2.0 ng/ml across 2, 4, and 16 $mg.m^{-3}$doses. Dopamine (DA) reached 4.8, 1.6 and 7.5 ng/ml in 2, 4 and 16 $mg.m^{-3}$doses at 4 hr. (T_{max} of PCBs in brain).At 4 hr corresponding to T_{max} of PCBs in brain, the brain 5-HT levels were 3.0, 2.6 and 4.3 $ng.ml^{-1}$ in 2, 4 and 16 $mg.m^{-3}$doses. Brain levels of Noradrenaline and Dopamine were reduced as PCB 77 concentrations reached maximum in brain. PCB 180 levels did not correlate with either central behavioral disruption or brain levels of all three monoamines.

Effects of Aroclor 1232 on brain monoamine levels: Relationship with behavioral scores:

Brain monoamine levels were estimated using HPLC-FLD in the first six hours for each dose level. Brain monoamine levels followed a linear relationship for 5-HT, exponential relationship for NA and non-monotonic or bimodular for DA in the first six hours of exposures. These could be correlated

with behavioral scores at C_{max} time points for each dose level (Figure 5, Table 4, Table 5).

Generally, a decline in mood was noted due to dipping of scores for grooming and restlessness sub-domains. The mean grooming scores across all days dipped at 5 hours for higher dose levels as compared to 4 and 6 hours for 2 $mg.m^{-3}$ dose. The trough in mean restlessness scores across all days was 3 hours for low dose and this shifted by one hour for each geometric increase in dose (Supplemental files 4A, 4B and 4C).

In 2 $mg.m^{-3}$exposed animals, the mood was generally depressed due to reduction in grooming at 3 and 4 hours, and increase in restlessness at 3, 4, and 5-hour time points. Grooming scores was back to control values at 5 hours in this dose, whereas increased restlessness did not recover even at 6 hours in this group. In the 4 mg/cu.m dose groups, grooming scores dipped at 3, 4, and 5-hour time points. But restlessness scores increased at 3, 4 and 5-hour time points. In the 16 $mg.m^{-3}$dose level, the grooming reduction was maximum at 3, 4, and 5 hr after exposure and did not recover even at 6 hr in this dose level. Restlessness was increased 3 hr, after which it came to normal at 4 hr time point in this dose level. At 5 hr after exposure, the restlessness scores increased, which did not recover back in this dose level.

In this study, the mean palpebral opening was below the normative value at all time points, with the maximum dip approached at 4 hours for 2 $mg.m^{-3}$dose and at 5 hours for higher doses. Although the heart rate was below the normative score during all time points, there was a tendency for maximum bradycardia at 3-5 hours across all dose levels. Similarly, the respiratory rate tended to be below the normative always and to initially dip at about 2-3 hours and then recover to baseline value at 5 hours across all days and dose levels. Urination scores increased at 5 hours for 2 and 4 $mg.m^{-3}$doses across the day averages, whereas it reduced at 4 and 5 hours for the 16 $mg.m^{-3}$dose.

Among the miscellaneous domains, group clustering (social behavior) activity increased with duration of exposure as the animals tended to reduce the surface area of their fur to avoid exposure to the potentially irritating PCBs. This was inversely proportional to the exploratory activity of mice in the chamber. Exploratory activity increased at 3, 4, and 5 hr in 2, 4, and 16 $mg.m^{-3}$dose levels. Social behavior increased at 3, 4, and 5 hr in 2 and 16 $mg.m^{-3}$dose levels. However, in the 4 $mg.m^{-3}$dose level, the social behavior scores were normal at 3 hr, then it decreased at 4 hr, after which it again recovered back to normal at 5 hr (Table 5).

Relationship of sub-domain behavior scores across doses:

The total behavioral subdomain scores across each time point compared pair wise during single 6-hour exposure to Aroclor 1232 exhibited dose dependence at 2.0, 4.0 and 16.0 $mg.m^{-3}$dose levels (F ratio = 18.41, $p < 0.0001$). The Pearson Correlation coefficients were close to 1.0 for most of the pair-wise comparisons. One-way Repeated Measures ANOVA of each correlation coefficients showed that the significance was due to inter-dose variation than inter-individual variability ($p = 0.0077$).

Dose dependence of sub-domain scores at T_{max}:

Likewise, the behavior scores were statistically significant at T_{max} of PCB 77 and PCB 180 (3-5 hours) with p value 0.0011. Friedman test of the correlation coefficients at T_{max} was significant across doses (Friedman statistic = 31.04). There was more of behavioral depression than stimulatory effects of PCBs at T_{max}.

Contribution of sub-domains to dose-dependent behavioral effects of PCBs at T_{max}:

Mood, which composed of five sub-domains namely grooming, restlessness, vocalization, irritability, and fearfulness, was generally depressed across all doses of exposures. This was generally ascribed to depression in grooming behavior on Irwin scale across all three doses, although restlessness seemed to increase dose-dependently. Concentrations of PCB 77 and PCB 180 in lung, brain and plasma significantly correlated negatively with mood depression at 6 hours in each dose level (-0.75 <Pearson 'r'< -0.85, Figure 6). Grooming depression was maximum at 4 hours and Restlessness was maximally increased at 4 hours across all doses of exposure (Figure 7).

Of the eleven autonomic system functions including palpebral opening, writhing response, pupil size, exophthalmos, pupil size, urination, salivation, hypothermia, skin colour, heart rate and respiratory rate, palpebral opening, urination and skin colour increased at 5 hours of exposure, especially at 4.0 and 16.0 $mg.m^{-3}$ doses. However, heart rate and respiration, reduced at 3 hours maximally across all three doses.

Out of the three miscellaneous domains (social aggregation, hostility, and exploratory activity), social conglomeration increased at 3-5 hours across all three doses, and exploratory activities increased at 3-5 hours across 2.0 and 16.0 $mg.m^{-3}$ dosing groups.

Generally, the peak of central behavioral disruption followed the T_{max} of PCB 77, whereas PCB 180 C_{max} contributed more to the peripheral effects on behavior (Table 4, and, Table 5, Supplemental files 1A, 1B, and 5).

Discussion

The Inhalation dosing of mice with Aroclor 1232 in the whole-body inhalation chamber was successful in producing significant plasma and brain concentrations of the two representative PCB congeners. The pharmacokinetic parameters of these two congeners in the two matrices were hence derived with high degree of correlation across the dose levels. Plasma peak concentrations of both congeners across all the three doses were generally one-and-half times greater than the brain levels. The time to peak absorption of both the congeners were nearly two times longer for PCB 77 than PCB 180, and this

was generally independent of dose and tissue matrix. Higher (nearly twice) AUCs were obtained for PCB 77 in both the matrices than PCB 180 generally across the three dose levels. The plasma AUC was generally higher than the brain AUC for both the congeners across the three dose levels, with dose proportionality.

PCBs are known to possess high orders of K_{ow} providing them with high lipophilicity and a capacitance to traverse across biological membranes [7]. PCBs can travel across biological barriers by both passive diffusion and active transport mechanisms. Hence the individual PCB congeners would be expected to cumulate and have longer biological half-lives. However, as is apparent from the acute inhalation study of Aroclor 1232, dosing PCB mixtures can impart only intermediately long half-lives (in hours) and MRTs for certain key toxic congeners like PCB 77 and even the hepta-CB, PCB 180, in plasma and brain tissue as well. This may also explain the short elimination rate constants, which are inversely correlated with log K_{ow}, and hence lipophilicity of congeners [13]. Though tetra-CB congeners like PCB 77 do not accumulate in aquatic fishes and other environmental reservoirs [14], certain high chlorinated congeners can attain significantly toxic concentrations and large AUCs in mouse and human matrices, as can be deduced from the large peak plasma and brain concentrations of PCB 180 and PCB 77, especially in higher doses. Such results have similarly been obtained with other investigations [15] where PCB 77 concentrations in different areas of the brain like the striatal cortex have been generically less compared to PCB 180 concentrations, after feeding rats with Aroclor 1254 in drinking water and chow. Although a dynamic equilibrium exists among the final tissue concentrations of homologs of PCBs as suggested by studies elsewhere [16] the concentrations of certain toxic congeners can significantly vary in same organism in different tissues [17]. In our study, both congeners generally have large volumes of distribution and clearance rates from both plasma and brain, which are dose-dependent and not time-of-exposure-dependent.

Many PCBs especially higher chlorinated PCBs like PCB 180 can have active metabolites in plasma and other tissues like methoxy, sulphated and hydroxy metabolites, which could shorten the elimination rate constants of such congeners *in-vivo* [18]. Hence, a change in rate of elimination of a congener from a tissue matrix with increments in dose usually depends on two factors – the rate of metabolism to active metabolites, and the rate of excretion versus distribution constants. Since PCB 180 is more lipophilic chlorinated and may have a greater number of potent active metabolites, its volumes of distribution and elimination half-lives change more drastically with dose increments, than PCB 77. Finally, for most of the pharmacokinetic parameters calculated for both congeners in this study, the variations imparted by the inhalation route is less, and the dose-dependent nature of their linear pharmacokinetics in brain and plasma is of a geometrically graded nature.

Since PCB 77 is more of a dioxin like PCB with ability to bind to AhRs and cause neuroendocrine disruption more than PCB 180, this study confirms the hypothesis that environmental reminisce of such PCBs, though seemingly low chlorinated can lead to monoamine and other neurotransmitter alterations in the brain even in rodents, via entry into the biological systems by inhalation routes. Further, such alterations could theoretically lead to neurobehavioral disruptions and neurological disorders if they accumulate in the brain as life progresses.

Lipophilicity of PCB congeners depends on number of chlorine atoms on the biphenyl nucleus and absorption of PCBs onto biological membranes in turn depends on lipophilicity and steric hindrance caused by position of chlorine on biphenyl congener [19, 20]. Ultimately absorption of PCBs in biological systems is influenced by the physical and stereo-chemical properties, and structure-activity and structure-metabolism relationships [21, 22]. PCBs are known to sequester into tissues richly supplied by blood flow like intestine wall and this occurs by passive diffusion [21, 22]. Since PCBs are known to get absorbed via oral, dermal, and inhalation routes, the main routes of excretion include, obviously, fecal, cutaneous, and respiratory air.

PCB congeners are known to accumulate in adipocytes, irrespective of dose and species, apart from blood - hence, their distribution *in vivo* is determined by the blood flow and lipophilicity directly [23, 24].Generally, ortho-substituted PCBs have higher partition coefficients as compared to non-ortho and dioxin-like PCBs which possess all forms of chlorine substitutions [25, 26]. PCB 77 being a dioxin-like highly lipid soluble PCB sequesters to brain but cannot be removed easily due to tissue sequestration in brain tissues and cause thyroid and neuroendocrine disruption [27, 28]. However, PCBs are known to disrupt Blood Brain Barrier and enter the brain easily on high doses [29, 30].

Hepatic binding to cytochrome enzymes also determines their higher hepatic concentrations. PCBs are known to induce the metabolism of other xenobiotics including PCBs themselves. Further fate and accumulation of PCBs are determined by what fraction of the administered congener is metabolized and how fast this process happens. This depends on chemistry of each congener [31]. Coplanar PCBs which have favorable spatial arrangement for hindering metabolism, and mono-ortho substituted PCBs, do not get metabolized easily [30, 32]. Unsubstituted chlorine at para-position changes the ability of the congener to form toxic arene metabolites easily causing free radical damage. [21, 33]. Non-substituted PCBs at ortho-meta positions and possession of higher number of chlorine atoms like PCB 180, makes the congener to cumulate, especially after higher or repeated dosing [25, 34, 35].

Elimination kinetics of PCB congeners vary between tissue compartments and the dose-exposure protocol. For example, the half-life of 3,3',4,4'-TCB was 1.07–2.60 days, since this congener sequesters in adipocytes, liver and thymocytes [19]. Congeners which do not tissue-cumulate, like 2,2',5,5'-TCB, have half-lives from 1.64–2.90 days. Since their concentrations were similar despite this difference in tissue sequestration, the property that decides elimination kinetics is therefore potency of congener, apart from chirality of stereochemistry [32]. PCB 77 being highly more potent than PCB 180 in its toxic effects, makes it selectively cumulate in brain and produce behavioral depression and shorter plasma elimination half-lives [36, 37].

The key results for behavioral effects of the PCB mixture include a decline in mood, autonomic and miscellaneous domain sub-scores, in a dose dependent and time dependent manner at each dose level, being contributed significantly by alteration in certain sub domains. The decline in grooming in this study at 2–4 hours after exposure initiation hence is not due to introduction of mice to novel environment of chamber and can be ascribed to be influenced only by the PCB induced neurotransmitter changes in brain. The negative chronotropic effects at 2 hours of exposure to PCBs may be due to cardiac, reflex, and

central depression. This is also the time when PCBs are about to redistribute from blood to the brain after being absorbed from the alveoli. Respiratory rate reduced at 4 hours of daily exposures especially in high dose exposures.

Generally, DA reduced at T_{max} for each dose level; 5-HT increased exponentially as T_{max} approached for each dose level; and NA followed U-shaped dose response, the dip being correlated at C_{max}. PCBs are known to affect monoamine levels in neurons inside and outside the Central Nervous System, via multiple mechanisms, including inhibition of VMAT-2 and reduction of dopamine levels in synapse [38]. In their ability to alter dopamine levels in brain and cause depression in humans, non-coplanar PCBs are more potent, as in this study wherein the decrease in dopamine and mood were maximum at PCB 77 T_{max}. The probable mechanisms of these behavioral effects of PCB 77 inhalation on mood are outlined in Fig. 7, which may be relevant in causing neuropsychiatric illnesses (e.g. major depression, autism, OCD) in human patients. In conclusion, this study proves that PCBs can alter behavior and monoamine levels via inhalation route.

Abbreviations

5-HT = 5-hydroxytrytamine; Acetonitrile (ACN); ANOVA= Analysis of Variance; AUC_{0-t} = Area-Under-Plasma-Concentration-time-Curve from time = 0 to time = t; $AUC_{0-\infty}$ = Area-Under-Plasma- Concentration-time-Curve from time = 0 to time = infinity; CB = Chloro-biphenyl; CPCSEA = Committee for the Purpose of Control and Supervision of Experimentation in Animals; C_{max} = Maximum Concentration; CL = Clearance; DA = Dopamine; DAD = Diode Array Detector; EDTA = Ethylene Diamine Tetra Acetate; FLD = Fluorescence detector; HPLC = High-Performance Liquid Chromatography; IAEC = Institutional Animal Ethics Committee; ID_{50} = Inhibitory Dose-50%; IS = Internal Standard; K_{el} = Elimination rate constant; K_{ow} = Log (Octanol-water) partition coefficient; OECD = Organization for Economic Cooperation & Development; MRT = Mean Residence Time; NA = Noradrenaline; PCBs = Polychlorinated Biphenyls; PBS = Phosphate Buffered Saline; QC = Quality Control; RM-ANOVA = Repeated Measures-Analysis of Variance; $t_{1/2}$ = Half-life; TBME = tertiary-Butyl Methyl Ether; T_{max} = Maximum Time; UPLC = Ultra-high Performance Liquid Chromatography; UV = Ultraviolet; V_d = Volume of Distribution.

Declarations

Ethical approval & Regulatory compliance statement:

The study was carried out after PSG-IAEC approval from a CPCSEA-approved Animal Facility. The study was conducted in accordance with CPCSEA guidelines, 2005.

Consent to participation: NOT available

Consent to publication: NOT available

Authorship contributions:

SSK contributed to manuscript editing in addition to data analysis and visualization and chromatography (new methods). NR contributed to study inception, design, data analysis, manuscript writing and editing and data analysis.

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Declaration of competing interest

The authors declare no conflicts of interests during the conception and inception of the paper, including any non-financial and monetary ones. All authors read and approved the manuscript, and all data were generated in-house and that no paper mill was used.

Data availability statement:

The manuscript includes data which is linked to the study results. This data collection can be made available on reasonable and justified request and shall be bound by the publishers' data management policy for submitting manuscripts involving original research. This data policy does not breach any confidentiality and is based sound ethical compliance. Infact the supplementary files have been attached for initial review and plausible publication, including review thereafter, to augment and enhance the clarity, reproducibility, and authenticity of the results discussed, with prior approval of all the authors.

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References

1. Stockholm Convention - Home page. http://www.pops.int/default.aspx. Accessed 25 Jan 2022
2. Health UD of, Services H (1999) Agency for Toxic Substances and Disease Registry-ATSDR.

3. Eriksson P, Fredriksson A (1996) Developmental neurotoxicity of four ortho-substituted polychlorinated biphenyls in the neonatal mouse. Environ Toxicol Pharmacol 1:155–165. https://doi.org/10.1016/1382-6689(96)00015-4
4. Seegal RF (1996) Epidemiological and laboratory evidence of PCB-induced neurotoxicity. Crit Rev Toxicol 26:709–737. https://doi.org/10.3109/10408449609037481
5. Simon T, Britt JK, James RC (2007) Development of a neurotoxic equivalence scheme of relative potency for assessing the risk of PCB mixtures. Regul Toxicol Pharmacol 48:148–170. https://doi.org/10.1016/j.yrtph.2007.03.005
6. Louis C, Covaci A, Crocker DE, Debier C (2016) Lipophilicity of PCBs and fatty acids determines their mobilisation from blubber of weaned northern elephant seal pups. Sci Total Environ 541:599–602. https://doi.org/10.1016/j.scitotenv.2015.09.094
7. Irwin S (1968) Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia 13:222–257. https://doi.org/10.1007/BF00401402
8. Control C for the P of, Animals S on E on (2003) CPCSEA Guidelines for laboratory animal facility. Indian J Pharmacol 35:
9. OECD (2009) OECD guidance document on acute inhalation toxicity testing. The Environment, Health and Safety Division, Organization for Economic Co …
10. Narayanan R, Muthusamy S, Lakshmi SV, et al (2021) Design and development of whole-body rodent inhalation chamber for exposure to Aroclor 1232 in Swiss albino mice. Int J Environ Sci Technol. https://doi.org/10.1007/s13762-021-03515-8
11. Ramanujam N, Sivaselvakumar M, Ramalingam S (2017) Fast and parallel determination of PCB 77 and PCB 180 in plasma using ultra performance liquid chromatography with diode array detection: A pharmacokinetic study in Swiss albino mouse. Biomed Chromatogr 31:. https://doi.org/10.1002/bmc.4000
12. Wang H, Walaszczyk EJ, Li K, et al (2012) High-performance liquid chromatography with fluorescence detection and ultra-performance liquid chromatography with electrospray tandem mass spectrometry method for the determination of indoleamine neurotransmitters and their metabolites in sea lamprey plasma. Anal Chim Acta 721:147–153. https://doi.org/10.1016/j.aca.2012.01.025
13. Grandjean P, Budtz-Jørgensen E, Barr DB, et al (2008) Elimination half-lives of polychlorinated biphenyl congeners in children. Environ Sci Technol 42:6991–6996. https://doi.org/10.1021/es800778q
14. Carpenter DO (2015) Exposure to and health effects of volatile PCBs. Rev Environ Health 30:81–92. https://doi.org/10.1515/reveh-2014-0074
15. Seegal RF, Okoniewski RJ, Brosch KO, Bemis JC (2002) Polychlorinated biphenyls alter extraneuronal but not tissue dopamine concentrations in adult rat striatum: an in vivo microdialysis study. Environ Health Perspect 110:1113–1117. https://doi.org/10.1289/ehp.021101113
16. Matthews HB, Dedrick RL (1984) Pharmacokinetics of PCBs. Annu Rev Pharmacol Toxicol 24:85–103. https://doi.org/10.1146/annurev.pa.24.040184.000505
17. Berninger JP, Tillitt DE (2019) Polychlorinated biphenyl tissue-concentration thresholds for survival, growth, and reproduction in fish. Environ Toxicol Chem 38:712–736. https://doi.org/10.1002/etc.4335
18. Grimm FA, Hu D, Kania-Korwel I, et al (2015) Metabolism and metabolites of polychlorinated biphenyls. Crit Rev Toxicol 45:245–272. https://doi.org/10.3109/10408444.2014.999365
19. Clevenger MA, Roberts SM, Lattin DL, et al (1989) The pharmacokinetics of 2,2',5,5'-tetrachlorobiphenyl and 3,3',4,4'-tetrachlorobiphenyl and its relationship to toxicity. Toxicol Appl Pharmacol 100:315–327. https://doi.org/10.1016/0041-008x(89)90317-7
20. Lotti M (2003) Pharmacokinetics and blood levels of polychlorinated biphenyls. Toxicol Rev 22:203–215. https://doi.org/10.2165/00139709-200322040-00003
21. Corrigan FM, Murray L, Wyatt CL, Shore RF (1998) Diorthosubstituted polychlorinated biphenyls in caudate nucleus in Parkinson's disease. Exp Neurol 150:339–342. https://doi.org/10.1006/exnr.1998.6776
22. Parham FM, Portier CJ (1998) Using structural information to create physiologically based pharmacokinetic models for all polychlorinated biphenyls. II. Rates of metabolism. Toxicol Appl Pharmacol 151:110–116. https://doi.org/10.1006/taap.1998.8441
23. Parham FM, Kohn MC, Matthews HB, et al (1997) Using structural information to create physiologically based pharmacokinetic models for all polychlorinated biphenyls. Toxicol Appl Pharmacol 144:340–347. https://doi.org/10.1006/taap.1997.8139
24. Kodavanti PR, Ward TR, Derr-Yellin EC, et al (1998) Congener-specific distribution of polychlorinated biphenyls in brain regions, blood, liver, and fat of adult rats following repeated exposure to Aroclor 1254. Toxicol Appl Pharmacol 153:199–210. https://doi.org/10.1006/taap.1998.8534
25. Oberg M, Sjödin A, Casabona H, et al (2002) Tissue distribution and half-lives of individual polychlorinated biphenyls and serum levels of 4-hydroxy-2,3,3',4',5-pentachlorobiphenyl in the rat. Toxicol Sci 70:171–182. https://doi.org/10.1093/toxsci/70.2.171
26. Beck V, Roelens SA, Darras VM (2006) Exposure to PCB 77 induces tissue-dependent changes in iodothyronine deiodinase activity patterns in the embryonic chicken. Gen Comp Endocrinol 148:327–335. https://doi.org/10.1016/j.ygcen.2006.04.003
27. Simmons SL, Cummings JA, Clemens LG, Nunez AA (2005) Exposure to PCB 77 affects the maternal behavior of rats. Physiol Behav 84:81–86. https://doi.org/10.1016/j.physbeh.2004.10.022
28. Jandacek RJ, Rider T, Keller ER, Tso P (2010) The effect of olestra on the absorption, excretion and storage of 2,2',5,5' tetrachlorobiphenyl; 3,3',4,4' tetrachlorobiphenyl; and perfluorooctanoic acid. Environ Int 36:880–883. https://doi.org/10.1016/j.envint.2009.06.010
29. Seelbach M, Chen L, Powell A, et al (2010) Polychlorinated biphenyls disrupt blood-brain barrier integrity and promote brain metastasis formation. Environ Health Perspect 118:479–484. https://doi.org/10.1289/ehp.0901334

30. Quinete N, Esser A, Kraus T, Schettgen T (2017) PCB 28 metabolites elimination kinetics in human plasma on a real case scenario: Study of hydroxylated polychlorinated biphenyl (OH-PCB) metabolites of PCB 28 in a highly exposed German Cohort. Toxicol Lett 276:100–107. https://doi.org/10.1016/j.toxlet.2017.05.025
31. Kania-Korwel I, Lehmler H-J (2016) Chiral polychlorinated biphenyls: absorption, metabolism and excretion–a review. Environ Sci Pollut Res Int 23:2042–2057. https://doi.org/10.1007/s11356-015-4150-2
32. Dhakal K, Gadupudi GS, Lehmler H-J, et al (2018) Sources and toxicities of phenolic polychlorinated biphenyls (OH-PCBs). Environ Sci Pollut Res Int 25:16277–16290. https://doi.org/10.1007/s11356-017-9694-x
33. Haraguchi K, Koga N, Kato Y (2005) Comparative metabolism of polychlorinated biphenyls and tissue distribution of persistent metabolites in rats, hamsters, and Guinea pigs. Drug Metab Dispos 33:373–380. https://doi.org/10.1124/dmd.104.002444
34. Danis B, Cotret O, Teyssié JL, et al (2004) Coplanar PCB 77 uptake kinetics in the sea star Asterias rubens and subsequent effects on reactive oxygen species (ROS) production and levels of cytochrome P450 immunopositive proteins (CYP1A-IPP). Marine Ecology Progress Series 279:117–128. https://doi.org/10.3354/meps279117
35. Kania-Korwel I, Lehmler H-J (2016) Toxicokinetics of chiral polychlorinated biphenyls across different species–a review. Environ Sci Pollut Res Int 23:2058–2080. https://doi.org/10.1007/s11356-015-4383-0
36. Tan Y, Song R, Lawrence D, Carpenter DO (2004) Ortho-substituted but not coplanar PCBs rapidly kill cerebellar granule cells. Toxicol Sci 79:147–156. https://doi.org/10.1093/toxsci/kfh108
37. Viluksela M, Heikkinen P, van der Ven LTM, et al (2014) Toxicological profile of ultrapure 2,2',3,4,4',5,5'-heptachlorbiphenyl (PCB 180) in adult rats. PLoS ONE 9:e104639. https://doi.org/10.1371/journal.pone.0104639
38. Bemis JC, Seegal RF (2004) PCB-induced inhibition of the vesicular monoamine transporter predicts reductions in synaptosomal dopamine content. Toxicol Sci 80:288–295. https://doi.org/10.1093/toxsci/kfh153

Tables

Pharmacokinetic Parameters	PCB 77					
	Plasma			Brain		
	2.0 mg/cu.m (mean±SD, N=4)	4.0 mg/cu.m (mean±SD, N=4)	16.0 mg/cu.m (mean±SD, N=4)	2.0 mg/cu.m (mean±SD, N=4)	4.0 mg/cu.m (mean±SD, N=4)	16.0 mg/cu.m (mean±SD, N=4)
C_{max}(ng/mL)	88.32±8.28	245.62±39.58	727.40±42.52	69.43±10.54	175.13±6.42	526.21±28.63
T_{max}(hr)	3.50±1.00	4.00±0.00	4.00±0.00	3.50±1.00	4.00±0.00	2.00±0.00
AUC_{0-t} (ng.hr/mL)	1321.19±55.72	2749.52±392.00	7565.21±148.44	1254.97±57.51	2366.40±142.19	6798.93±340.47
$AUC_{0-\infty}$ (ng.hr/mL)	1626.02±64.13	2957.28±413.20	8013.39±263.11	1469.62±161.80	2581.12±136.46	7100.56±413.72
$T_{1/2}$ (hr)	20.17±1.38	12.72±0.36	11.17±1.54	16.20±4.36	14.20±0.58	10.82±1.29
MRT_{last}	16.51±0.42	14.31±0.49	14.29±0.24	17.07±1.93	15.05±0.32	14.39±0.30
K_{el} (hr^{-1})	0.03± 0.00	0.06±0.00	0.06±0.01	0.05±0.01	0.05±0.00	0.07±0.00
Vd ($ml.m^{-2}$)	35858.25±3073.55	25209.01±3835.06	32099.78±3593.04	31481.64±5927.66	31856.13±3012.73	35070.00±2110.32
CL ($ml.hr^{-1}m^{-2}$)	1231.46±49.43	1375.07±215.15	1998.28±65.96	1373.32±150.80	1553.12±85.97	2258.90±127.06

Table 1: The Mean Pharmacokinetic Parameters of PCB 77 in Plasma and Brain after inhalation of PCBs at 2, 4 and 16 mg/m^3 dose

Pharmacokinetic Parameters	PCB 180					
	Plasma			Brain		
	2.0 mg/cu.m (mean±SD, N=4)	4.0 mg/cu.m (mean±SD, N=4)	16.0 mg/cu.m (mean±SD, N=4)	2.0 mg/cu.m (mean±SD, N=4)	4.0 mg/cu.m (mean±SD, N=4)	16.0 mg/cu.m (mean±SD, N=4)
C_{max}(ng/mL)	66.49±5.22	370.21±42.59	829.57±31.40	42.04±4.83	135.49±4.95	429.35±14.37
T_{max}(hr)	2.00±0.00	2.00±0.00	6.00±0.00	2.00±0.00	2.00±0.00	6.00±0.00
AUC_{0-t} (ng.hr/mL)	763.27±38.80	4369.75±730.55	13192.39±498.68	631.12±46.60	2449.37±186.79	8332.99±123.54
$AUC_{0-\infty}$ (ng.hr/mL)	1226.52±136.72	5425.39±408.95	15636.03±1053.10	915.48±106.63	3896.91±367.99	10984.01±590.37
$T_{1/2}$ (hr)	28.95±3.39	20.04±6.43	18.21±1.94	22.17±3.24	31.43±3.10	22.73±3.57
MRT_{last}	13.11±0.96	15.43±0.85	16.24±0.32	13.88±0.21	19.45±0.64	18.72±0.21
K_{el} (hr^{-1})	0.02±0.00	0.04±0.01	0.04±0.01	0.03±0.01	0.02±0.00	0.03±0.01
Vd ($ml.m^{-2}$)	68083.41±1151.95	21729.82±8734.97	26825.47±1287.33	69838.36±5306.26	46934.13±6867.21	47593.68±4968.74
CL ($ml.hr^{-1}m^{-2}$)	1646.03±184.38	740.51±57.37	1026.94±72.53	2207.04±256.46	1033.24±96.14	1459.71±75.49

Table 2: The Mean Pharmacokinetic Parameters of PCB 180 in Plasma and Brain after inhalation of PCBs at 2, 4 and 16 mg/m^3 dose

Dose	Concentration in Plasma (mean ± SD, ng/mL)	Concentration in Brain (mean ± SD , ng/g)	Brain / Plasma Ratio
PCB 77			
2 mg/ m^3	88.32 ± 8.28	277.72 ± 42.17	3.17 ± 0.53
4 mg/ m^3	245.62 ± 39.58	700.53 ± 25.68	2.91 ± 0.50
16 mg/ m^3	727.40 ± 42.52	2104.83 ± 114.52	2.90 ± 0.22
PCB 180			
2 mg/ m^3	66.49 ± 5.22	168.16 ± 19.33	2.56 ± 0.46
4 mg/ m^3	370.21 ± 42.59	541.94 ± 19.81	1.48 ± 0.12
16 mg/ m^3	829. 57 ± 31.40	429.35 ± 14.37	0.52 ± 0.03

Table 3: Brain to plasma ratio of PCB 77 and PCB 180 in Swiss albino mouse after inhalation of PCBs at 2, 4 and 16 mg/m^3 dose

5-HT (ng/ml)	2 mg/cu.m		4 mg/cu.m		16 mg/cu.m	
	Mean	SD	Mean	SD	Mean	SD
2 hour	2.07	0.07	2.11	0.13	3.08	0.23
4 hour	3.04	0.27	2.62	0.22	4.32	0.47
6 hour	4.33	0.67	3.29	0.23	4.36	0.18
DA (ng/ml)	2 mg/cu.m		4 mg/cu.m		16 mg/cu.m	
2 hour	2.99	0.54	1.75	0.45	3.39	1.04
4 hour	2.42	0.64	0.83	0.13	3.73	0.48
6 hour	5.15	0.85	4.91	0.51	5.07	0.46
NA (ng/ml)	2 mg/cu.m		4 mg/cu.m		16 mg/cu.m	
2 hour	0.24	0.08	0.27	0.16	0.23	0.15
4 hour	1.88	0.25	0.85	0.11	1.01	0.09
6 hour	5.28	0.67	5.13	1.35	2.15	0.15

Table 4: Tabulation of mean monoamine concentrations at T_{max} after exposure to different doses of Aroclor 1232

BEHAVIOURAL DOMAIN	BEHAVIOURAL SUB-DOMAIN EFFECTS OF PCB 77 AT 4 Hrs. (4 mg/cu.m of AROCLOR 1232)
AWARENESS	
	ALERTNESS
	VISUAL PLACING
	PASSIVITY
	STEREOTYPY
MOOD	
	GROOMING
	VOCALIZATION
	RESTLESSNESS
	IRRITABILITY / AGGRESSION
	FEARFULNESS
MOTOR ACTIVITY	
	REACTIVITY TO ENVIRONMENT
	SPONTANEOUS ACTIVITY
	TOUCH RESPONSE
	PAIN RESPONSE
CNS EXCITATION	
	STARTLE RESPONSE
	STRAUB TAIL
	TREMORS
	TWITCHES
	CONVULSIONS
POSTURE	
	BODY POSTURE
	LIMB POSITION
MOTOR INCOORDINATION	
	STAGGERING GAIT
	ABDOMINAL GAIT
	RIGHTING REFLEX
MUSCLE TONE	
	LIMB TONE
	GRIP STRENGTH
	BODY SAG
	BODY TONE
	ABDOMINAL TONE
REFLEXES	
	PINNA
	CORNEAL
	IFR (Ipsilateral flexor Reflex)
AUTONOMIC	

	WRITHING
	PUPIL SIZE
	PALPEBRAL OPENING
	EXOPHTHALMOS
	URINATION
	SALIVATION
	PILOERECTION
	HYPOTHERMIA
	SKIN COLOUR
	HEART RATE
	RESPIRATORY RATE
MISCELLANEOUS	
	EXPLORATORY ACTIVITY
	HOSTILITY
	SOCIAL BEHAVIOR
LETHALITY / DEAD	ACUTE
	DELAYED

Colour-coding:

No change absolutely in all animals	**CNS Modulatory change in all animals**	**CNS depression in 50% animals**	**CNS excitation in 50% animals**
Mild change in 50% of animals	**CNS modulation in 50% animals**	**CNS depression in all animals**	**CNS excitation in all animals**

Table 5: Behavioral effects of 4 mg/cu.m dose level of Aroclor 1232 at PCB 77 T_{max} in brain.

Figures

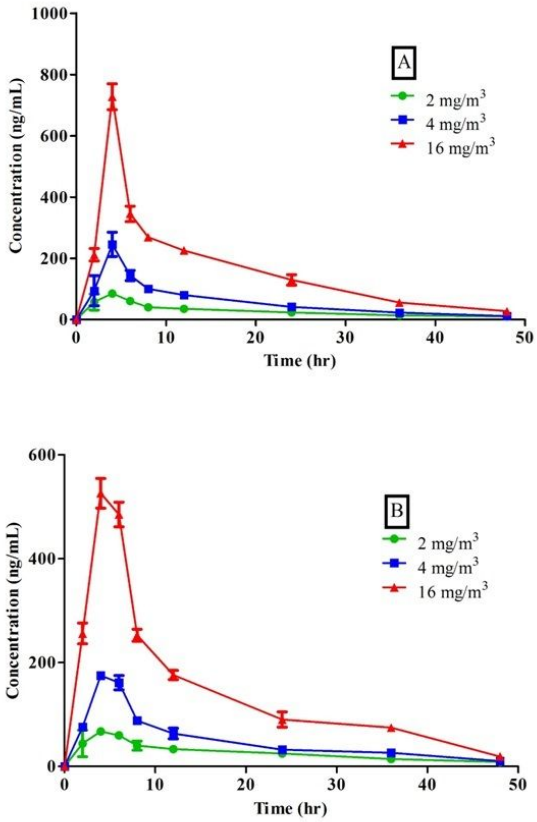

Figure 1: Inset A - Mean plasma concentration–time profile of PCB 77 in mouse plasma after inhalation of PCBs at 2, 4 and 16 mg/m^3 dose. Inset B - Mean tissue concentration–time profile of PCB 77 in mouse brain after inhalation of PCBs at 2, 4 and 16 mg/m^3 dose.

Figure 1

Inset A - Mean plasma concentration–time profile of PCB 77 in mouse plasma after inhalation of PCBs at 2, 4 and 16 mg/m^3 dose. Inset B - Mean tissue concentration–time profile of PCB 77 in mouse brain after inhalation of PCBs at 2, 4 and 16 mg/m^3 dose.

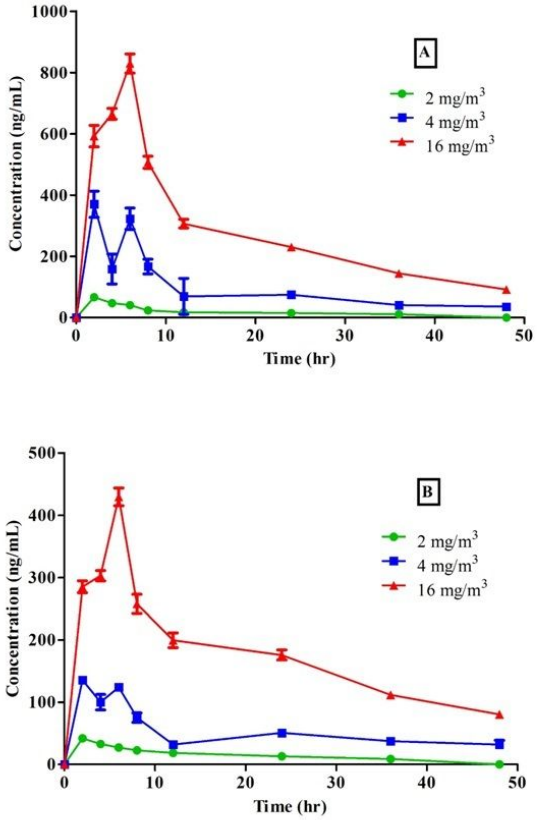

Figure 2: Inset A - Mean plasma concentration–time profile of PCB 180 in mouse plasma after inhalation of PCBs at 2, 4 and 16 mg/m^2 dose. Inset B - Mean tissue concentration–time profile of PCB 180 in mouse brain after inhalation of PCBs at 2, 4 and 16 mg/m^2 dose.

Figure 2

Inset A - Mean plasma concentration–time profile of PCB 180 in mouse plasma after inhalation of PCBs at 2, 4 and 16 mg/m^3 dose. Inset B - Mean tissue concentration–time profile of PCB 180 in mouse brain after inhalation of PCBs at 2, 4 and 16 mg/m^3 dose.

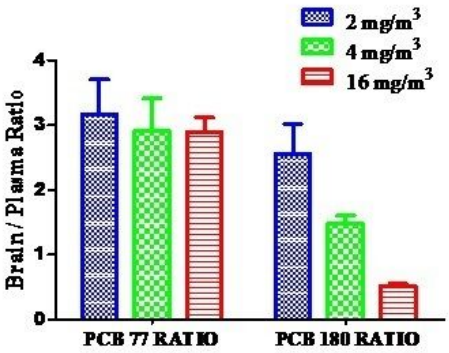

Figure 3: Dose dependent comparison of Brain: plasma ratios of PCB 77 and PCB 180

Figure 3

Dose dependent comparison of Brain: plasma ratios of PCB 77 and PCB 180.

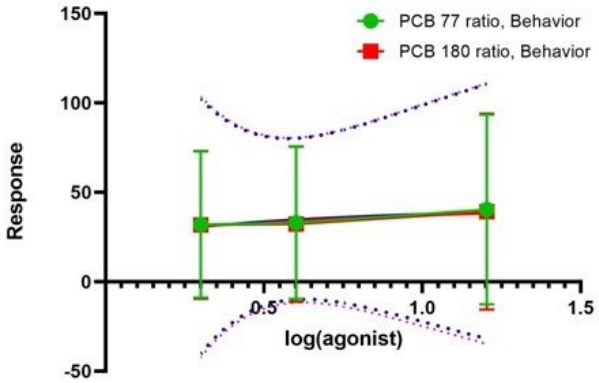

Figure 4: Hill model showing ID-50 hyperbolas for PCB 77 and PCB 180 brain to plasma ratios, and behavioral depression (PCB 77) / stimulation (PCB 180).

Figure 4

Hill model showing ID-50 hyperbolas for PCB 77 and PCB 180 brain to plasma ratios, and behavioral depression (PCB 77) / stimulation (PCB 180).

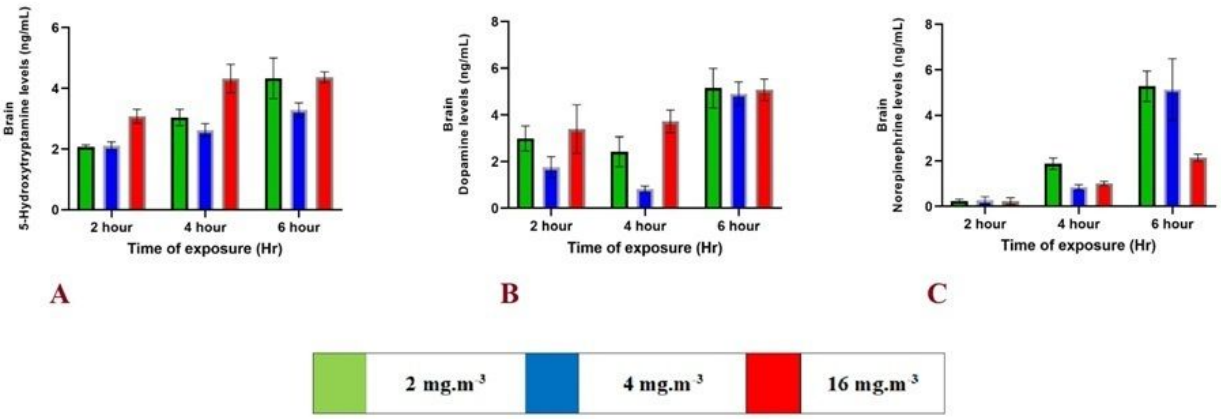

Figure 5: Dose response graphs for brain monoamine levels, relating these effects of PCBs at T_{max}: inset A for 5-HT, inset B for DA, inset C for NA.

Figure 5

Dose response graphs for brain monoamine levels, relating these effects of PCBs at T_{max}: inset A for 5-HT, inset B for DA, inset C for NA.

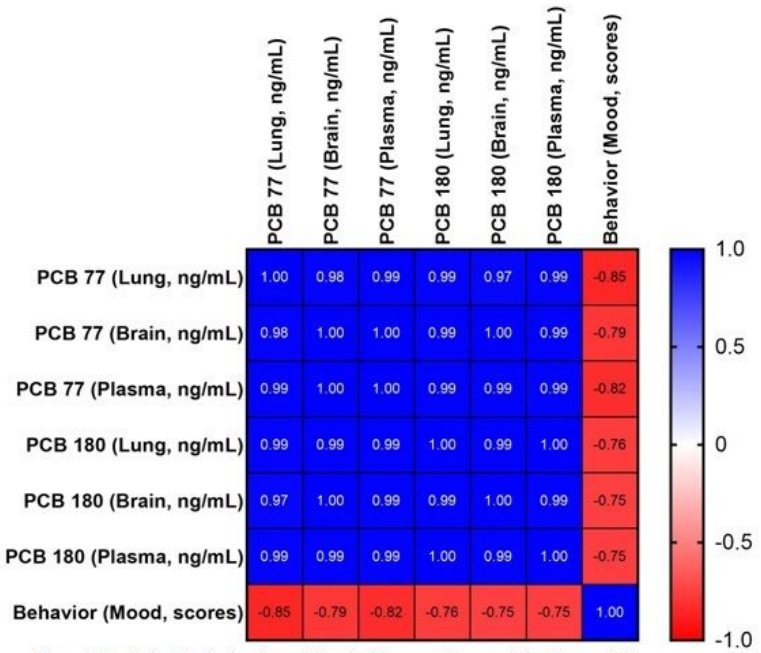

Figure 6: Correlation Matrix showing pairwise significant negative correlation between PCB congener concentrations in lungs, brain and plasma, and mood scores at 6 hours of exposure to each dose of Aroclor 1232.

Figure 6

Correlation Matrix showing pairwise significant negative correlation between PCB congener concentrations in lungs, brain and plasma, and mood scores at 6 hours of exposure to each dose of Aroclor 1232.

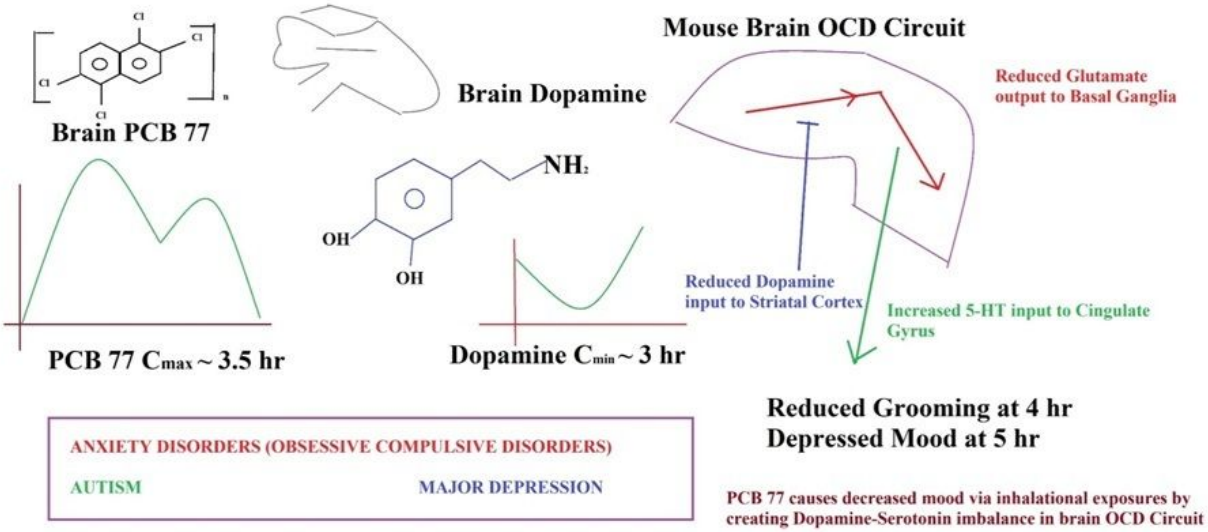

Figure 7: Mechanistic toxicology of PCB 77 on mood changes in Swiss albino mouse, elaborating the toxicokinetic-toxicodynamic mechanism of PCB toxicities on mood.

Figure 7

Mechanistic toxicology of PCB 77 on mood changes in Swiss albino mouse, elaborating the toxicokinetic-toxicodynamic mechanism of PCB toxicities on mood.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplemental1A.xlsx
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- Supplemental2A.xlsx
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- Supplemental4B.pzfx
- Supplemental4C.pzfx
- Supplementalfile5.pzfx