

Low Doses of a PCB (Aroclor 1254) Affect the Body Weight by Decreasing the Activity of Glucose-6-Phosphatase in the Liver and Kidney Cells of Mice

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Abstract : In this communication, toxicity of very low doses of Aroclor 1254, the most potent PCB, was evaluated in respect to gross body weight and glucose-6-phosphatase (G6Pase) activity in liver and kidney cells of mice. The study was designed to test three hypotheses. Groups of adult male Swiss albino mice were subjected to two very low and environmentally available doses (0.1 & 1 mg/kg bw/d) of Aroclor 1254 exposed for three durations (4, 8 & 12 d). The toxicant was administered orally and the specific activity of glucose-6-phosphatase was estimated from the liver and kidney cells of mice. The doses selected were significantly lower than the lethal dose but comparable to possible human exposure from different sources. The results revealed significant dose and exposure duration dependent effects of Aroclor on total body weight and the activity of glucose-6-phosphatase in liver and kidney cells of mice. The observed results suggested that all three hypotheses were true. It may be possible that the activation of AhR mediates the suppression of body weight and the activity of glucose-6-phosphatase, which suggested a role for these pollutants as disruptors of energy metabolism.

Keywords - Aroclor 1254, Glucose-6-phosphatase, Mice, Liver, Kidney.

I. INTRODUCTION

Polychlorinated biphenyls (PCBs) are one of the major persistent organic pollutants (POPs). There are 209 PCB congeners are categorized based on the number of chlorine atoms in the compound. A common classification divides PCBs into dioxin-like and nondioxin-like based on their structural and toxicological similarity with the dioxin molecule [1]. These compounds are physically, thermally and chemically stable and thus, used in a variety of industrial applications [2]. PCBs have the tendency to bioaccumulate, bioconcentrate and biomagnify in the tissues of humans and animals which is a matter of serious concern. Because of improper and careless waste disposal properties living beings are easily exposed to this compound [2]. Exposure to PCBs is responsible for variety of adverse effects such as wasting syndrome, immunotoxicity, skin disorders, neurological, metabolic, endocrine and reproductive effects in both humans and animals [2]. Some of these effects have been confirmed by laboratory experiments and include progressive weight loss ('wasting syndrome'), alteration of lipid metabolism, hepatotoxicity, immunotoxicity, alteration of endocrine system function and reproduction, teratogenicity and developmental toxicity, and tumor promotion [3- 8]. The mechanism of toxicity indicate that some of the PCB congeners act by the same mechanism as the chlorinated dioxins i.e., the toxicity is probably facilitated through the interaction with the Ah receptor. One of the major devastating effects of AHR activation by TCDD is lethal wasting syndrome in which decreased liver gluconeogenesis and body weight are prominent features [9, 10]. It produces the starvation-like state in which gluconeogenesis and food intake decreased. Therefore, the aim of the present study was to evaluate the *in vivo* toxic effect of different sublethal doses and exposure durations of Aroclor 1254 on body weight and the activity of glucose-6-phosphatase in liver and kidney cells of mice. The study was designed to test three hypotheses (a) toxicity of Aroclor 1254 suppresses the body weight of mice due to inhibition in the activity of glucose-6-phosphatase, (b) if so, than it will exhibit a typical dose-duration response and, (c) since the functionality of G6Pase is maximum in liver, toxic effect of Aroclor 1254 will be more in liver than kidney.

II. Materials and Methods

2.1 Reagents

The PCB used in this study, Aroclor 1254, was procured from Sigma-Aldrich Chemicals Ltd. (CAS No. 11097-69-1). All other chemicals and reagents were of analytical grade and also procured from the Sigma Chemical Co. (St. Louis, MO, USA) for the assessment of glucose-6-phosphatase in liver and kidney of mice.

2.2 Animals

Male Swiss albino mice (30-40 g) were kept in the animal house facilities at prescribed conditions as per CPCSEA, India. Animals were given commercially available rodent diet and water *ad libitum*. Different animal groups for the experiments were kept in a standardized conditions (26 ± 2 °C; 44–55 % RH, 10 : 14 h light and dark cycles) for one week before the experiment [11]. All studies were conducted according to the ethical norms approved by the CPCSEA, India (CPCSEA/CH/RF/ACK-2003, 29-07-2003).

2.3 Experimental design and treatments

A total of 63 adult male Swiss albino mice were used for the study. The selection of the doses were based on the (a) available reports of the doses causing non-carcinogenic effects in the liver and kidney tissue of mice, especially on the enzymes (acute to sub-acute exposure), and (b) evaluation of toxicity studies and application of factors (LOAEL) for extrapolating from animal model to human for Aroclor 1254 administered through oral route [12]. The doses selected therefore, were very low concentrations of Aroclor 1254, comparable to that of a possible human exposure from different environmental sources. Different groups of mice were given oral administration of Aroclor 1254 (0.1 and 1 mg/kg body weight /d) dissolved in corn oil (vehicle) for three different exposure durations of 4, 8 and 12 days.

2.4 Tissue preparation

After completion of toxic exposure, the liver and kidney was rapidly removed and washed in ice-cold Sucrose – EDTA - Imidazole buffer (SEI buffer). Known amount of tissue was homogenized using Potter-Elvehjem glass homogenizer to make a 10% (w/v) tissue concentration. The tissue preparation and enzyme extraction procedure were as per the method of Zaugg [13] with appropriate modifications.

2.5 Evaluation of Glucose-6-phosphatase activity

Activity of Glucose-6-phosphatase was estimated by the method of Shimeno [14] with some appropriate modifications [15] and inorganic phosphate was measured by the method of Fiske and Subbarow [16]. To calculate the specific activities of the enzymes studied, protein content of each sample was estimated as per the method of Lowry et al. [17] using Folin phenol reagent and bovine serum albumin as the standard.

2.6 Statistical analyses

The obtained data were subjected to various statistical analyses for their cumulative acceptability and for testing the hypotheses formulated. Comparison between control and doses were made using one-way ANOVA. A two-way nested ANOVA was done to check the significance in the variations between different doses and amongst different exposure durations. In addition to those tests, Comparison for the significance variations between control and each durations within a given dose were performed using two-tailed Student's 't' test. All statistical procedures were done as per Sokal and Rohlf [18].

III. Results and Discussion

Results of the present study showed noticeable reduction in the body weight of mice in all the doses and exposure durations. However, more effect was observed in higher dose and exposure duration (Fig.1). Similar results were also observed in the specific activity of glucose-6-phosphatase. It showed a clear dose and duration dependent inhibitory trend in liver and kidney cells of mice for both the doses and exposure durations. Specific activity of glucose-6-phosphatase showed high inhibition in both the doses (0.1 & 1 mg/kg bw/d) of Aroclor 1254 in all the exposure durations (4, 8 & 12 days) but less effects were observed after 4 days of exposure duration (Fig. 2a). In case kidney cells, the specific activity of G6Pase showed the inhibitory trend while, greater inhibition was observed after 12 days exposure (Fig. 2b). Activity glucose-6-phosphatase in liver showed greater inhibition than the kidney.

Dioxins or dioxin-like PCBs have diverse toxic effects all of which require binding to the AHR [19, 20, 21, 22]. These effects generally include unregulated nutrient metabolism and energy production, immune system dysfunction, carcinogenesis, and cardiac contractile dysfunction [9, 10, 23-25]. PCB-mediated inhibition of gluconeogenesis potentially threatens glucose homeostasis and energy metabolism in exposed humans and animals. PCB-induced disruptions in gluconeogenesis were reported to promote hypoglycemia in susceptible patients during fasting [26]. In human body, only two organs viz., liver and kidney possess sufficient glucogenic enzyme activity and glucose-6-phosphatase activity enabling them to release glucose into the circulation by gluconeogenesis [27]. Glucose-6-phosphatase is widely considered as a key enzyme involved in systemic glucose homeostasis [28, 29]. The results of this study exhibited a general inhibitory trend in the enzyme activity in liver and kidney of mice. Results of statistical analyses showed the dose and duration dependent effects on the gross body weight and G6Pase enzyme activity. Body weight of mice showed the significant dose

and duration dependent reduction, as well as an inhibitory trend in the activity of glucose-6-phosphatase in both the selected tissues (Fig. 1, 2 a & b). No significant variations were observed within each group (Fig. 1, 2 a & b). Results of two-factor ANOVA showed the exposure duration dependent significant variations in the body weight and activity of glucose-6-phosphatase in liver and kidney, whereas, highly significant variations in the activity of glucose-6-phosphatase were observed in liver cells than kidney (Table-1). The observed results pointed out the possibility of impaired gluconeogenesis and associated decrease in body weight, a possible indicative of wasting effect. It was obvious from the present investigation that both doses of Aroclor 1254 had the immediate effects on the activity of glucose-6-phosphatase but higher dose showed more effect in the enzyme activity than the lower dose. Results of Student's t-test showed highly significant variations in the activity of glucose-6-phosphatase between exposure durations within each dose (Table 2). Similar kind of results was also observed in one-way ANOVA between individual exposure duration within each group (Table 3). All three statistical analyses showed the significant variations in the body weight of mice as compared to control (Table- 1, 2 & 3). In mammalian liver, TCDD is known to decrease the expression and activity of PEPCK and G6Pase, enzymes that mainly control gluconeogenic flux [10, 30-32]. However, the exact mechanism for these effects remains obscure [19, 20]. This effect of PCB raises the new possibility that the AHR may contribute the disturbances in glucose homeostasis.

The present investigation reports dose and exposure duration dependent decline in the body weight and specific activity of glucose-6-phosphatase greater in liver than kidney cells of mice after *in vivo* PCB exposure. As the majority of the studies pertaining to the metabolic effects of dioxin exposure use animal models mostly rodents, broad strain and species specificity demonstrates the sensitivity to wasting effect of dioxins [33, 34, 30, 35-38]. Many observations across such studies include impaired gluconeogenesis, decrease in body weight, and reductions in the glucose-6-phosphatase activity and gene expression of PEPCK [35, 32, 40, 10, 39]. In the given broader distribution, and environmental contamination by, PCBs, compared to TCDD [41,42], the effects of PCB on hepatic and renal glucose metabolism are potentially important.

IV. Figures and Tables

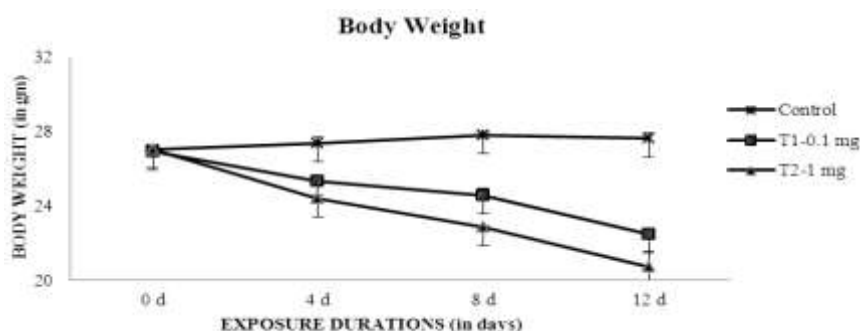


Fig. 1. Graphs showing dose and duration dependent alterations in the body weight after *in vivo* Aroclor 1254 intoxication. Error bars represent the standard deviation and “*” sign represents the significant variations at P = 0.05 level in the body weight of mice.

TABLE 1. Result of Two-Factor ANOVA between control and toxicated groups in liver and kidney of the adult male albino mice. The results of two-factor ANOVA showed the significant time dependent toxic effects of Aroclor 1254.

	Body Wt.	Liver	Kidney
Amongst doses	1.27	1.21	0.76
Within durations	23.25*	19.90*	24.73*

* Significant at P = 0.05 ($F_{crit} (df = 3, 8) = 3.01$)

* Significant at P = 0.05 ($F_{crit} (df = 8, 35) = 2.36$)

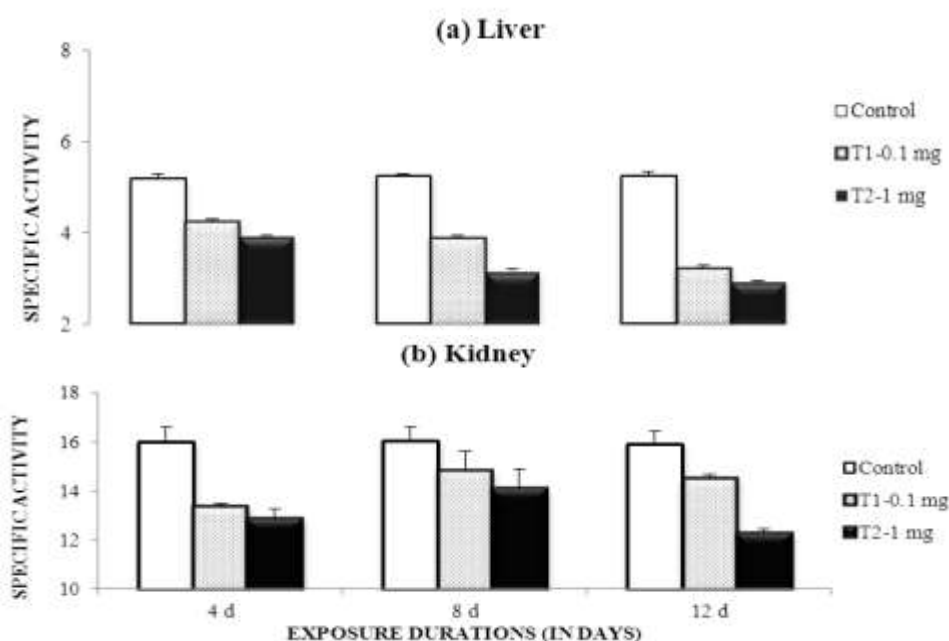


Fig. 2. Graphs showing the dose and duration dependent alterations in the specific activity of Glucose-6-phosphatases after Aroclor 1254 intoxication. The error bars represents the standard deviation and “*” sign represents the significant variations at P = 0.05 level in the activity of Glucose-6-phosphatases in tissues concern.

TABLE-2. Result of t-test between control and individual exposure duration within each dose in liver and kidney of the adult male albino mice. The statistical analysis showed that all the selected tissues were significantly affected by the Aroclor 1254 intoxication.

	Body Wt.		Liver		Kidney	
	0.1mg	1mg	0.1mg	1mg	0.1mg	1mg
4DAYS	11.88*	17.41*	25.23*	37.78*	7.69*	7.83*
8DAYS	14.55*	50.67*	79.15*	16.22*	2.38	3.82*
12DAYS	33.12*	16.51*	44.52*	54.63*	4.92*	12.36*

*Significance at P = 0.05 ($T_{crit.} = 2.447, df = 6$)

TABLE -3. Result of Single factor ANOVA between individual exposure durations within each group in liver and kidney of the adult male albino mice. Results showed the significant variations in the selected tissues in both the doses.

	Body Wt.	Liver	Kidney
CONTROL	2.85	0.60	0.04
0.1MG	16.73*	11.41*	9.43*
1MG	60.23*	29.48*	10.84*

*Significance at P = 0.05 ($F_{crit.} = 4.26, df = 2, 9$)

V. CONCLUSIONS

The overall results of the present study showed that even a very low dose (10,000 and 1000 times lower dose than the LD₅₀ value for mice) of Aroclor 1254 had the effect on the body weight of mice and on the activity of glucose-6-phosphatase in liver and kidney of mice. This finding answers the hypotheses that (a) toxic effect of Aroclor 1254 did decrease the body weight of mice which was possibly due to inhibition in the activity of glucose-6-phosphatase, (b) the observed effects on the body weight and activity of glucose-6-phosphatase was a dose and exposure duration dependent response and, (c) toxic effect of Aroclor 1254 was more significantly inhibited the activity of glucose-6-phosphatase in liver than kidney. One of the major possibilities of direct toxic effect of PCB was that it activated the AhR receptor which ultimately lead to the wasting

syndrome in which decreased liver gluconeogenesis and body weight are noticeably effected. This situation produced the starvation-like state in which gluconeogenesis and food intake decreased.

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