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Alterations in the Activities of Few Lysosomal Enzymes in the Kidney and Intestine of Mice Exposed to Low Concentrations of a PCB, Aroclor 1254 under *in vitro* condition

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Abstract: *Polychlorinated biphenyls (PCBs) are industrial POPs which have been released into the environment resulting in widespread and persistent contamination. The aim of the present investigation was to determine the alterations in the activities of few lysosomal enzymes (acid phosphatase, α-galactosidase, β-galactosidase and β-glucuronidase) in lysosomal cellular sub-fractions of kidney and intestine of mice after the exposure to different concentrations of Aroclor 1254 (25 µg/ml, 50 µg/ml and 100 µg/ml) for two different time intervals (15 and 30 minutes). The study was aimed to answer a couple of hypothesis set in null form, that (a) Aroclor 1254 does not have any direct effect on the selected lysosomal enzymes under in vitro condition, and (b) Aroclor 1254 does not exhibit any organ-specific toxic effects on the activities of selected lysosomal enzymes in kidney and intestine of mice under in vitro condition. The overall results of the present study revealed that the even very low concentration of Aroclor 1254 has direct and organ-specific toxic effects on the activities of selected lysosomal enzymes in kidney and intestine of mice, cancelling both the hypotheses. Therefore, the results of present study were suggested that Aroclor 1254 have the ability to induce the lysosomal destabilization by altering the activities of different lysosomal enzymes which may lead to apoptosis or necrosis in different tissues of mice.*

Key words: *Aroclor 1254, Intestine, Kidney, Lysosomal enzymes, PCBs.*

I. Introduction

PCBs are widespread in environment through sewage outfalls and industrial disposal into water ways [1]. In addition to this, the long half-life of these POPs has led to detectable levels in the adipose tissue of a large proportion of the human population [2]. PCBs are formed by chlorination of biphenyls and are complex mixtures containing isomers of chlorobiphenyls with different chlorine content [3]. Different PCB congeners have different rates of persistence, bioaccumulation and toxicity [4]. In general, as the number and position of chlorine increases, the rate of metabolism and detoxification decreases [5]. Lysosomes are membrane bound organelle and their functional role is intracellular digestion and recycling of macromolecules like proteins, carbohydrates and lipids [6]. The lysosome also plays an important role in the immune system and the overall health of organisms. For instance, decreased cellular digestion of macromolecules may result in reduced growth of the organisms, supporting that extended lysosomal destabilization caused by continuous exposure to toxic chemicals such as PAHs, PCBs and metals can adverse effects at higher biological levels like organ or individual [7]. However, stabilized lysosomes are essential for the health of organisms. In a tiered approach, disturbances in the activities of lysosomal enzymes, which are the indicator of lysosomal destabilization, can used as an early warning screening tool to identify chemical exposure and potential adverse biological effects before they are manifested as more overt biological responses commonly measured in environmental assessment like slanted growth or reproductive failure [8]. Therefore, the purpose of the present study was to elucidate the in vitro toxic effects of different concentrations of Aroclor 1254 on the activities of renal and intestinal lysosomal enzymes at different time intervals. The study tested two hypotheses set in null form, (a) Aroclor 1254 does not have any direct effect on the purified enzymes under *in vitro* condition, and (b) Aroclor 1254 does not exhibit any organ-specific toxic effects on the activities of selected lysosomal enzymes in kidney and intestine of mice under *in vitro* condition.

II. Materials And Methods

Healthy inbred male Swiss Albino mice, around 3 months of age and weighing 30 ± 5 g, were used for the entire study. The animals were fed with commercially available rodent diet and water *ad libitum*, and kept in the animal house facilities under hygienic condition as per CPCSEA India, guidelines. Humidity and temperature were controlled (25 ± 2°C) and diurnal cycle of 14:10 h was maintained. All experiments were conducted according to norms and approval of CPCSEA, India (CPCSEA/CH/RF/ACK-2003, 29-07-2003). One

of the PCBs, Aroclor 1254 in its purest form, was obtained from Sigma Aldrich Chemicals Pvt. Ltd. (CAS No. 1746-01-6) and used as toxicant. Whole kidney and middle portion of the small intestine of mice were taken as enzymes source. Three different concentrations of Aroclor 1254 viz., 25 µg/ml, 50 µg/ml and 100 µg/ml were prepared separately by dissolving desired amount of Aroclor 1254 in Dimethyl Sulphoxide (DMSO). Known amount of whole kidney and intestine tissue were sampled from the tissues of animal and homogenized in chilled phosphate buffer (pH 7.0) to obtain a 10% (w/v) homogenate. Enzyme extract preparation for purified lysosomal enzymes was carried out by the method of Beaufay [9]. Homogenate was centrifuged at 2000 rpm for 8 min at 4˚C. The obtained supernatant was re-suspended in phosphate buffer and centrifuged at 11,000 rpm for 40 min to isolate the lysosomal fraction. The resultant sediment was re-suspended in phosphate buffer with 0.1% Triton X 100 to obtain a supernatant of lysosomal fraction. The activity of Acid Phosphatase, α-galactosidase, β-galactosidase and β-glucuronidase were estimated using this lysosomal fraction extract. The enzyme assay was done as per the method of Tettamanti and Masserini [10]. However, before adding the respective substrate, enzyme aliquot was separately pre-incubated with selected concentrations (25 µg/ml, 50 µg/ml and 100 µg/ml) of Aroclor 1254 at room temperature for 15 and 30 minutes. For the estimating the specific activities of the selected enzymes, protein concentration of the tissue homogenate was estimated by the Lowry et al [11], using bovine serum albumin as standard. Enzyme assay without Aroclor 1254 served as control. The obtained data were subjected to various statistical analyses like one-way and two-way nested ANOVA and Student's 't' test for their cumulative acceptability and hypothesis testing as per Sokal and Rohlf [12].

III. Results And Discussion

Results of the present study showing the time interval dependent alterations in the specific activities of the lysosomal enzymes in kidney and intestine of mice after exposed to the different concentrations of Aroclor 1254 for different time intervals under the *in vitro* condition. In case of kidney, the inhibitory trend was observed in the activities of all the selected lysosomal enzymes. The gradual inhibition was observed from lower concentration to higher concentration of Aroclor 1254 in both the given time intervals as compared to control. Whereas, in case of intestine, the specific activities of Acid Phosphatase, α-galactosidase, β-galactosidase and β-glucuronidase were also inhibited after the exposure of different concentrations of Aroclor 1254 for 15 and 30 minutes. On the other hand, higher inhibition by the Aroclor 1254 was observed on the specific activity of β-galactosidase as compared to control. Therefore, the results indicated that the activities of selected lysosomal enzymes were not much disturbed after exposed to low concentration of Aroclor 1254 for 15 minutes. However, considerable effects on the lysosomal enzyme activities were observed in moderate to high concentration of Aroclor 1254 after 30 minutes of exposure duration.

Kidney and intestine are the two most essential excretory organs in mammals. Kidney participates in whole body homeostasis while small intestine (duodenum, ileum and jejenum) participates in chemical digestion, absorption of nutrients and also supports the body's immune system. The duodenum receives Brunner's glands, biliary and pancreatic secretions with bicarbonate which neutralises the acidic outflow of the stomach. The mucosal villi and microvilli covering the enterocytes give a vastly increased area for both secretory and absorptive activity along the whole of the small intestine [13]. In some of the functions of kidney and intestine, lysosomal enzymes have the important role especially in chemical digestion after the absorption of required nutrients. Lysosomes are cytoplasmic organelles and play an important role in a wide variety of physiological activities including intracellular digestion of macromolecules and toxic chemicals such as PAHs, PCBs and metals [14, 15]. PCBs and Dioxin like compounds are known to induce the alterations in the activities of lysosomal enzymes [16, 17]. In the present investigation, alterations in the activities of lysosomal enzymes after the exposure to different concentrations of Aroclor 1254 for various time-intervals were observed. Results showed the time-interval dependent toxic effects of Aroclor 1254 in the activities of selected lysosomal enzymes in kidney and intestine of mice. Specific activities of acid phosphatase and α- galactosidase showed high significant alterations in the intestine while the specific activities of β-galactosidase and β-glucuronidase showed high significant alterations in the kidney of mice than the intestine (Table-1). This sequestration of toxic chemicals may reduce the cell injury at low concentrations, whereas excess concentrations are reported to affect the lysosome, resulting in cell damage followed by cell death due to the loss of hydrolytic enzymes [18].

Results of single-factor ANOVA showed significant alterations in the activities of all selected lysosomal enzymes of kidney and intestine after exposure to different concentrations of Aroclor 1254 for various time-intervals. Acid phosphatase showed significant variations in 50µg/ml concentration in both the tissues. Similar results were observed in the activity of α- galactosidase in kidney but in intestinal tissue, the activity altered in 25µg/ml concentration. However, the activities of β-galactosidase and β-glucuronidase in kidney showed changes in 100µg/ml concentration of Aroclor 1254 whereas, the activity β-galactosidase altered in 50µg/ml concentration of Aroclor 125 and the activity of β-glucuronidase altered after the 25µg/ml exposure of Aroclor 1254 (Table-2). These alterations in the activities of the lysosomal enzymes are indicative of

alterations in cellular energy or metabolic requirements or direct effects of PCBs which is able to cause changes in size, quantity or membrane liability of lysosomes [19, 20, 17].

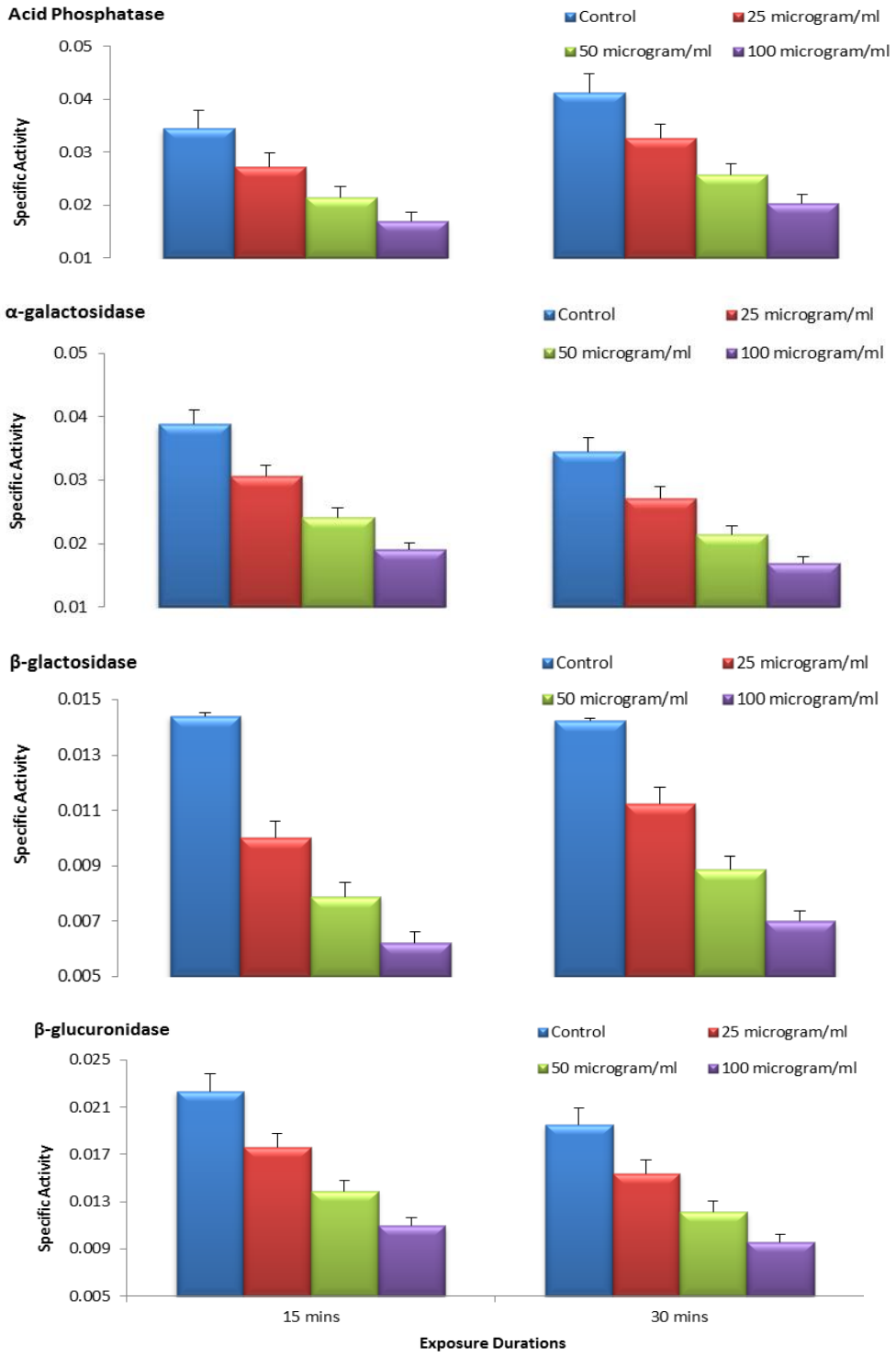

Fig. 1. Dose and duration dependent variations in the specific activities of few lysosomal enzymes from the kidney tissue of mice after *in vitro* exposure of Aroclor 1254.

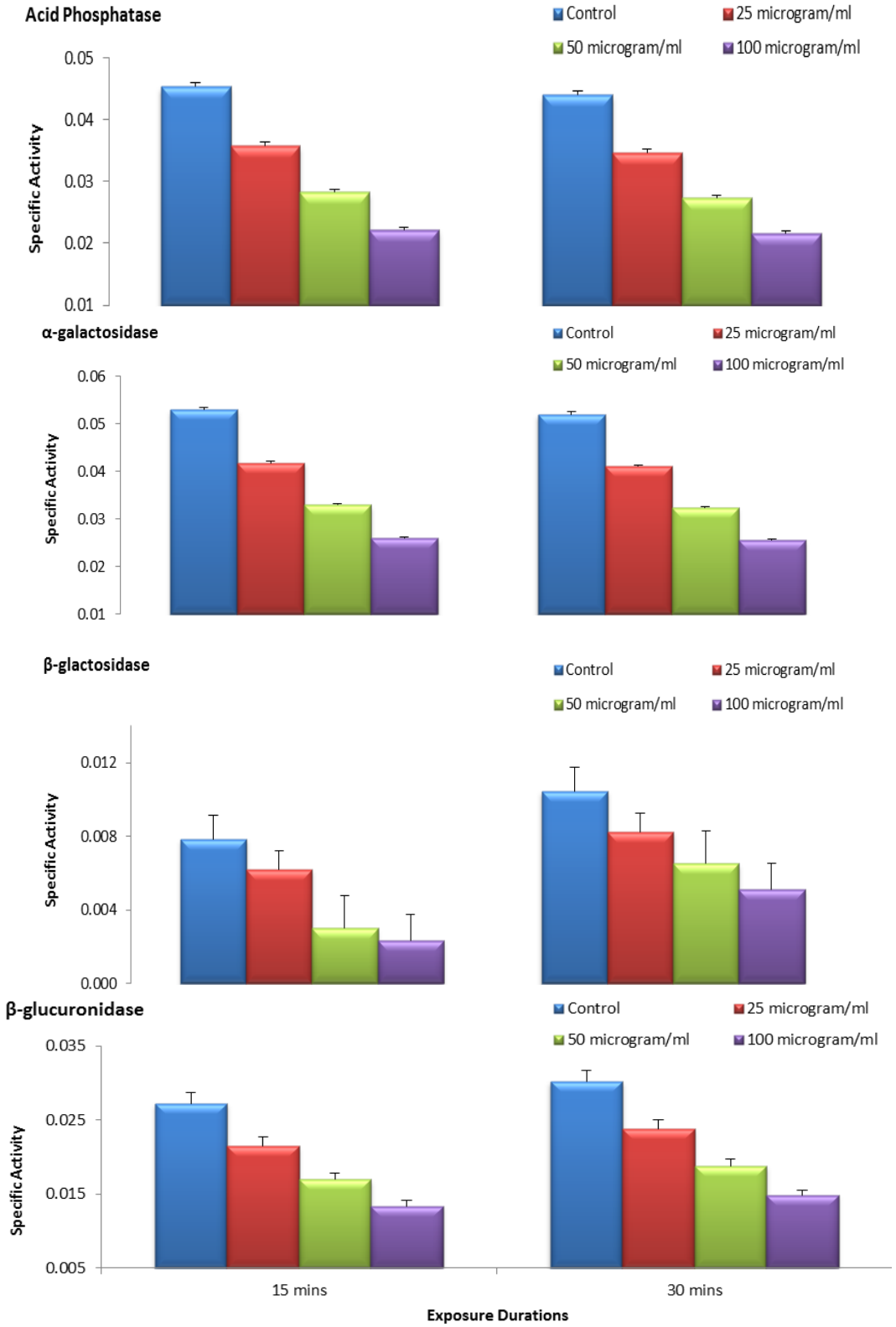

Fig. 2 Dose and duration dependent variations in the specific activities of few lysosomal enzymes from the intestine tissue of mice after *in vitro* exposure of Aroclor 1254.

Interestingly, when the obtained results were analyzed with the help of Student's t-test, significant alterations was observed in the activities of all the selected lysosomal enzymes in the kidney and intestine of mice. The specific activity of acid phosphatase in kidney showed the maximum changes after the exposure of 25 µg/ml concentration of Aroclor 1254 for 15 minutes and 50 µg/ml concentration of Aroclor 1254 for 30 minutes. However, the activities of α-galactosidase, β-galactosidase and β-glucuorindase showed the similar kind of

results. Disturbances in the activities of these enzymes were observed after the exposure of 50 µg/ml concentration of Aroclor 1254 for 30 minutes and 100 µg/ml for 15 minutes in the kidney of mice. However, in case of intestine, specific activities of acid phosphatse and β-galactosidase showed the similar kind of alterations. Activities of these enzymes were significantly altered after the exposure to 50 µg/ml of Aroclor 1254 for 15 minutes and 100 µg/ ml for 30 minutes. While the activity of α-galactosidase showed the significant alterations after the exposure of 25 µg/ml concentration of Aroclor 1254 for 30 minutes and 50 µg/ml for 15 minutes. But, the significant change was observed in the activity of β-glucuorindase after the exposure of 100 µg/ml Aroclor 1254 for 15 and 30 minutes time interval (Table-3). In many laboratory studies, disturbances in the lysosomal enzymes increased as body burdens increased were observed when exposed to PCBs [21]. However, the overall results of the present study answer the hypothesis that under *in vitro* condition, the lysosomal enzymes have the direct and organ-specific toxic effects of Aroclor 1254 [17, 22]. Therefore, the results of the present *in vitro* study suggested that even very low concentrations of PCBs also have the ability to affect various lysosomal enzymes directly and alter their specific activity [23], which can cause the lysosomal destabilization and leads to apoptosis or necrosis in *in vivo* condition.

Table-1. Result of Two-Factor ANOVA between control and toxicated groups in kidney and intestine of mice.

	Acid Phosphatase		**α-galactosidase**		**β-glactosidase**		**β-glucuronidase**	
	KIDNEY	**INTESTINE**	**KIDNEY**	**INTESTINE**	**KIDNEY**	**INTESTINE**	**KIDNEY**	**INTESTINE**
Amongst doses	0.66	0.97	1.73	0.27	0.83	4.26	0.97	0.59
Within durations	28.91*	59.99*	10.22*	40.34*	52.96*	36.78*	68.04*	21.79*

* Significant at P = 0.05 (*F crit (df=3, 7) = 3.07)*
** Significant at P = 0.05 (*F crit (df=7, 31) =2.48)*

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Table -2. Result of Single factor ANOVA between individual exposure durations within each group kidney and intestine of mice.

	Acid Phosphatase		**α-galactosidase**		**β-glactosidase**		**β-glucuronidase**	
	KIDNEY	**INTESTINE**	**KIDNEY**	**INTESTINE**	**KIDNEY**	**INTESTINE**	**KIDNEY**	**INTESTINE**
Control	24.27*	82.86*	53.22*	21.71*	0.58	43.42*	11.08*	10.87
25µg	27.68*	51.03*	10.45*	16.07*	13.09*	26.49*	23.78*	91.19
50µg	88.92*	51.91*	35.63*	6.42*	22.74*	98.49*	15.56*	15.78
100µg	26.89*	10.24*	4.69	13.25*	60.63*	17.05*	74.24*	0.09

*Significant at P = 0.05 (*F crit.* = 5.98)

Table-3. Result of t-test between control and individual exposure duration within each dose.

Kidney

	Acid Phosphatase			**α-galactosidase**			**β-glactosidase**			**β-glucuronidase**		
	25µg	**50µg**	**100µg**	**25µg**	**50µg**	**100µg**	**25µg**	**50µg**	**100µg**	**25µg**	**50µg**	**100µg**
15 minutes	14.81*	14.69*	12.52*	10.49*	8.33*	12.17*	29.63*	32.57*	33.75*	12.06*	7.95*	16.65*
30 minutes	7.60*	48.00*	12.80*	12.56*	21.44*	4.33*	9.56*	18.39*	9.17*	7.29*	13.78*	1.64

Intestine

	Acid Phosphatase			**α-galactosidase**			**β-glactosidase**			**β-glucuronidase**		
	25µg	**50µg**	**100µg**	**25µg**	**50µg**	**100µg**	**25µg**	**50µg**	**100µg**	**25µg**	**50µg**	**100µg**
15 minutes	0.21	14.49*	2.00	28.90*	43.23*	15.55*	45.93*	113.26*	34.32*	10.56*	13.09*	65.21*
30 minutes	30.57*	32.51*	38.46*	40.48*	6.62*	38.69*	19.71*	21.12*	27.59*	10.56*	13.09*	14.69*

*Significant at P = 0.05 (*T crit.* = 2.44)

IV. Conclusions

The present study revealed a predominantly time-interval dependent effects of the Aroclor 1254 on the few selected lysosomal enzymes in kidney and intestine of mice. However, the alterations in the lysosomal enzymes activity were clearly indicative of a direct and organ-specific effects of PCB in *in vitro* conditions. The overall results suggest that these alterations in the activities of lysosomal enzymes may cause destabilization of the lysosome as a whole in *in vivo* condition.

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