MUTAGENICITY OF POLYCHLORINATED PHENOXYPHENOLS (PREDIXOIN) AND THEIR PHOTO-DEGRADATION PRODUCTS

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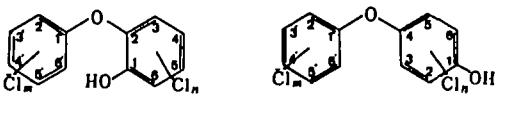
1. Introduction

Polychlorinated phenoxyphenols (PCPPs) occur as the main contaminant of technical chlorophenol formulation [1]. Attention has been drawn to such compounds because their 2-hydroxyl isomers (predioxins) have been to undergo both thermal and photochemical ring closure to form polychlorinated dibenzo-p-dioxins (PCDDs) [2]. Lamparski et al. [3] and Weerasinghe et al. [4] have however demonstrated the presence of PCDDs in manicipal sewage sludges. Our recent findings have also demonstrated that phenolic compounds react with chlorine in water to form PCPPs [5-8]. Although Deinzer et al. [9] have been reported that certain PCPPs may be as toxic as the most toxic PCDDs, no studies on genetic toxicity of these PCPPs are published. The data present here show the mutagenic effects of several PCPPs, their chlorination and photo-irradiation products, in water employing Salmonella/microsome assays.



Predioxin Isopredioxin

Fig. 1. Chemical Structures of Polychlorinated Phenoxyphenols

2. Experimental

5-Chloro-2-(2,4-dichlorophenoxy)phenol (Irgasan DP 300 = $C_{12}H_7Cl_3O_2$) was commercially available (purity, >99.3%). Several PCPPs were prepared according to the method described in the previous papers [7,8]. Several chlorinated phenols, which are expected to be formed during photo-irradiation in the absence and presence of chlorine in water, were also commercially available. Standard solutions of these compounds both *alone* and as mixtures were prepared by diluting the compounds in methanol and subsequent dilutions. Hypochlorous solution was prepared

by diluting NaOCl solution (c.a., 10% available Cl, Nacalai Tesque) in distilled water and were adjusted to pH 7 by addition of 0.1 M phosphate buffer solution. The hypochlorite concentrations were determined by iodometric titration.

Photo-irradiation of aqueous Irgasan DP 300 and related PCPP solutions (10 mg/l) was conducted in the absence and presence of chlorine (20 mg/l), utilizing a Rikokagaku Sangyo UVL-100 HA lamp. The lamp was equipped with a pyrex glass Jacket for cooling at 20℃ and UV light with maximum wave lengh of 365 nm was delivered at an intensity of approximately 4 mW /cm^2/s at the surface of the water jacket. 200 ml of Irgasan DP 300 or related PCPP solutions of pH 7 was introduced in a reaction vessel and exposed to the UV-light for desired times. The organic residues in the irradiated samples were extracted with diethyl ether (50 ml x 2) for gas chromatographic (GC) determinations and mutagenicity tests. A dark control experiment was performed by covering the reaction vessel with aluminum foil, to establish the extent of changes in the original compounds caused by the other elements of the procedure.

Tester strains of TA98 and TA100 of the Salmonella/microsome assay were employed essentially as described by Ames et al.[10] with minor modification. Characteristic properties of the bacteria were verified for each fresh stock and their mutagenic properties were again verified using positive and negative controls as part of each experiemnt. For testing mutagenesis requiring microsomal activation, polychlorinated biphenyl mixture-induced rat liver microsomal 9000 x g supernatant fraction (S9 mix) was utilized. The criterion for detection of mutagenesis in test samples was dose-dependent response exceeding the zero response (sponaneous control value) by at least two-fold.

A Shimadu GC-6A gas chromatograph equipped with a flame ionization detector and a 2 m x 3 mm I.D. glass column packed with 2% OV-1 on Uniport HP(60-80 mesh) was programmed fro 120 to 240℃ at 5℃/min. Other GC operating conditions were the same as described in the previous papers[7,8]. A Shimadu Model Chromatopac-1A data system was used to determine the retention times and peak areas on the chromatograms. An Hitachi M-80 combined mass spectrometer-gas chromatograph equipped with an Hitachi M-003 data processing system was used for the qualitative analyses of samples. The same separation column and operating conditions as described above were employed. The peaks appearing on the chromatograms were identified by comparison of their retention times and mass spectra with those of authentic compounds.

3. Results and Discussion

Table 1 shows the mutagenic effects of Irgasan DP 300 and related PCPPs on S. typhimurium TA98 and TA100 strains with and without S9 mix.

Table I. Mutagenic Effects of Polychlorophenoxyphenols (PCPPs) on Salmonella typhimurium TA98 and TA100 with and without S9 Mix

Compound[a] tested	Dose/ plate (μg)	Mutagenicity ratio[b] TA98		TA100		Compound tested	Dose/ plate (μg)	Mutagenicity ratio TA98		TA100	
		-S9	+S9	-S9	+S9			-S9	+S9	-S9	+S9
Irgasan DP 300	1	Tox[c]	0.86	Tox	1.07	2-Propyl-PCPPs	3	1.16	1.23	1.02	1.10
	10	Tox	Tox	Tox	0.50		30	1.08	1.10	1.05	0.97
	50	Tox	Tox	Tox	Tox		300	0.88	1.20	0.79	1.02
PCPPs	1	1.21	1.06	1.20	1.04	4-Propyl-PCPPs	3	1.10	1.15	1.20	1.00
	10	0.88	1.10	1.15	1.20		30	1.05	1.04	1.40	1.20
	50	Tox	0.99	0.72	1.10		300	1.10	1.00	1.60	1.15
2-Methyl-PCPPs	3	0.96	0.89	1.20	1.10	4-Butyl-PCPPs	3	1.24	1.20	1.07	1.13
	30	0.76	0.98	1.00	0.95		30	1.18	1.02	1.05	1.10
	300	0.80	0.93	0.68	1.20		300	0.99	1.20	Tox	1.00
4-Methyl-PCPPs	3	0.88	0.95	1.20	1.12	4-Pentyl-PCPPs	3	1.18	1.20	1.31	1.20
	30	1.20	1.16	2.70	1.15		30	1.04	1.07	1.00	1.15
	300	1.01	1.20	10.50	1.29		300	1.05	1.20	Tox	1.20
2-Ethyl-PCPPs	3	1.25	1.12	1.30	1.28	4-Hexyl-PCPPs	3	1.19	1.30	1.24	1.10
	30	1.05	1.15	1.00	1.14		30	1.22	1.10	1.00	1.08
	300	0.88	1.15	0.92	1.24		300	0.68	1.20	Tox	1.00
4-Ethyl-PCPPs	3	0.87	1.12	0.98	1.00	4-Heptyl-PCPPs	3	1.02	1.20	1.05	1.00
	30	1.20	1.10	1.90	1.20		30	1.22	1.08	1.00	1.15
	300	0.78	1.22	2.73	1.17		300	0.89	1.21	Tox	1.25
Spontaneous (DMSO)		18	25	118	125			18	25	118	125
2-Nitrofluorene(2.5 ug)		505	-	-	-			505	-	-	-
Sodium azide (1 ug)		-	-	780	-			-	-	780	-
Benzo(a)pyrene (5 ug)		-	489	-	763			-	489	-	763

a) These compounds were tested for mutagenicity in the ranges from 1 μg to 1000 μg per plate but no responses were detected, except for 4-Methyl-PCPPs, 4-Ethyl-PCPPs and 4-Propyl-PCPPs.
b) Average of 2 independent testes with 3 plates per dose in each test. Mutagenicity ratio = number of revertants per spontaneous revertants.
c) Toxic effect to the tester strain.

TOX

These PCPPs tested in this work were composed of the following proposed molecular formulae: $C_{12}H_7Cl_3O_2$ (13%), $C_{12}H_6Cl_4O_2$ (29%) and $C_{12}H_5Cl_5O_2$ (58%) for PCPPs; $C_{14}H_{11}Cl_3O_2$ (21%), $C_{14}H_{11}Cl_3O_2$ (43%) and $C_{14}H_{10}Cl_4O_2$ (36%) for 4-methylated PCPPs; $C_{15}H_{13}Cl_3O_2$ (21%), $C_{15}H_{13}Cl_3O_2$ (41%) and $C_{15}H_{14}Cl_4O_2$ (38%) for 4-ethylated PCPPs; $C_{16}H_{15}Cl_3O_2$ (40%), $C_{16}H_{15}Cl_3O_2$ (49%) and $C_{16}H_{16}Cl_4O_2$ (11%) for 4-propylated PCPPs and so on.

No mutagenic effects of original Irgasan DP 300 and PCPPs prepared by chlorination of simple phenol were observed, because they exhibited strong toxicity to the tester strains of TA98 and TA100 with and without S9 mix. 4-Methylated PCPPs prepared by the chlorination of p-cresol in aqueous solution were, however, mutagenic on TA100 in the absence of the rat liver homogenates (S9 mix). The presence of enzymatic activation with S9 mix reduced the activity of 4-methylated PCPPs on TA100 strain. Mutagenicity of 4-ethylated PCPPs and 4-propylated PCPPs were also detected on the same strain without S9 mix, but their activities were very low and decreased with an increase in the lengh of alkyl chains on the aromatic rings, as compared with that of 4-methylated PCPPs. 4-Butylated, 4-pentylated, 4-hexylated and 4-heptylated PCPPs were not mutagenic on both TA98 and TA100 strains with and without S9 mix. No mutagenic effects of 2-methylated PCPPs, 2-ethylated PCPPs and 2-propylated PCPPs on both TA98 and TA100 strains with and without mammalian activation system.

Irgasan DP 300, PCPPs and 4-methylated PCPPs were shown to be comparatively stable in aqueous solution of pH 7 under the dark condition. However, these compounds were rapidly converted to higher chlorinated PCPPs in water in the presence of chlorine, followed by the decomposition of these intermediates to chlorophenols. A rapid decrease in the amounts of original PCPPs in aqueous solution occurred when these PCPP solutions were exposed to photo-irradiation. The decomposition of original PCPPs, these intemediate PCPPs and chlorophenols formed in aqueous solution were accelerated under the photo-irradiation in the presence of chlorine and complete decompositions were observed for 5 hrs. These findings indicate that disappearance of vacterial toxicity and mutagenicity of original PCPPs may occur in aqueous solution under the photo-irradiation in the absence and presence of chlorine.

Figure 2 shows the mutagenic effects of diethyl ether extracts of aqueous Irgasan DP 300, PCPP and 4-methylated PCPP solutions (pH 7 at 20℃), after photo-irradiation in the absence and presence of chlorine for 5 hrs. As can be seen in Fig. 2A and 2B, vacterial toxicity of Irgasan DP 300 and PCPPs decreased after these solutions were exposed to chlorine alone and photo-irradiation alone. However, the PCPP solution exhibited mutagenicity to TA100 strain without S9 mix, after treatment with photo-irradiation in the presence of chlorine (Fig. 2B). In contrast to the Irgasan DP 300 and PCPP solutions, mutagenic effects of

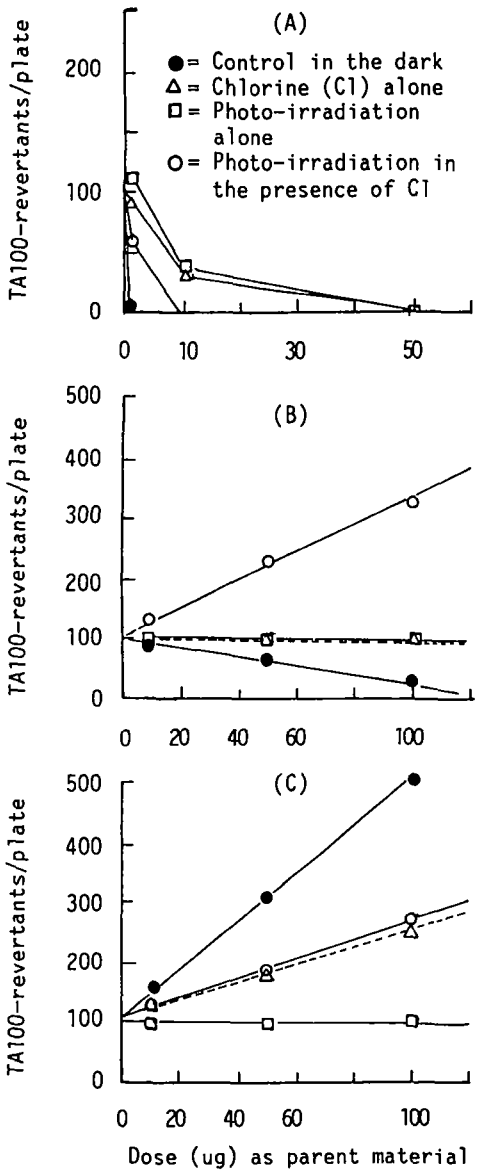


Fig. 2. Mutagenic Effects of Irgasan DP 300 (A), PCPPs (B) and 4-Methylated PCPPs (C), after Photo-irradiation in the Presence and Absence of Chlorine in Water (pH 7) for 5 hrs at 20°C

4-methylated PCPPs solution on TA100 strain without S9 mix were shown to be remained about 50%, after treatment with chlorine alone and photo-irradiation in the presence of chlorine (Fig. 2C). These findings indicate that some new mutagenic compounds from non-mutagenic PCPPs and mutagenic 4-methylated PCPPs are formed in aqueous solution under the photo-irradiation in the presence of chlorine.

4. References

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