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Residual Piperonyl Butoxide in Agricultural Products

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Piperonyl butoxide, α-[2-(2-Butoxyethoxy)ethoxy]-4,5-methylene-2-propyltoluene (PB), has been applied as a synergist of pyrethrin insecticide in arable fields and in storehouses of agricultural products. Production of pyrethrin insecticide containing PB in Japan was 57 tons in 1972 and 1973 respectively, and 36 tons in 1974.

PB shows the inhibitory effect on drug metabolism of insects and enhances the killing ability of insecticide (SAWICKI 1962; CASIDA 1970). It was also observed in vivo and in vitro that PB affected on the mixed-function oxidase system of mammalian livers (KISFALUDY et al. 1971; CONNEY et al. 1972; FRIEDMAN et al. 1972; PHILPOT and HODGSON 1972). FISHBEIN et al. (1972) reported that PB injected into rats was excreted at once. However, in the experiment of feeding rats with PB, GOLDSTEIN et al. (1973) observed that liver weight and microsomal protein were increased more than those of normal rats. They also observed the change of liver parenchymal cells with an electron microscope.

Maximum acceptable daily intake of PB by WHO is 0.03 mg/kg body weight/day. An investigation was carried out about residual PB in rices by KAWANA et al. (1976). They reported that PB was not detected from 33 samples of rices. The objective of this study was to determine the residual levels of PB in rices and other agricultural products.

MATERIALS AND METHODS

Samples The samples of this investigation were unhulled rice, hulled rice, wheat, barley, buck wheat, rye, oats, milo, corn, soy bean and red bean. Foreign samples were obtained from the Japan Grain Inspection Association. Concerning to rices, their harvested prefectures were shown in Table 1. All samples were kept at low temperature (below 5°C) until they were tested.

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TABLE 1

Harvested Prefectures of Tested Rices

Prefecture	Unhulled rice	Hulled rice
Fukuoka	28	14
Kumamoto	16	12
Oita	12	12
Yamaguchi	6	3
Miyazaki	5	3
Shimane	4	3
Hiroshima	3	0
Okayama	2	1
Niigata	2	4
Fukui	2	1
Unknown	28	17

Figures show the amount of tested samples.

Reagents PB standard for the pesticide analysis was supplied by Gas Chro Ind. Co. LTD.. Reagent grade n-hexane, ethanol and acetonitrile were redistilled in all glass system. The solvent was previously tested that it gave no interfered peak on a high speed liquid chromatogram. Reagent grade anhydrous sodium sulfate, sodium chloride and thymol were obtained from Wako Pure Chem. Ind. Co. LTD.. Anhydrous sodium sulfate was heated at 600°C for 2 hours prior to use.

Preparation of samples for analysis The sample was powdered (28 mesh) with a Wiley Mill (Yoshida Ind. Co., 1029A model). For extraction of PB, powdered sample (25 g) in a 300 ml separating funnel was shaken vigorously for 1 hour with 100 ml of n-hexane with a mechanical shaker (Iwaki Ind. Co., V-S model). After filtration with a glass filter (G-4) by suction, the residue was washed twice with fresh 50 ml of n-hexane. In the case of oats it was so difficult to filter by suction that centrifugation should be done (5000 r.p.m., 5 minutes).

Gathered filtrate or supernatant was shaken with 50 ml of acetonitrile previously saturated with n-hexane. The partition of n-hexane-acetonitrile was carried out 3 times. After the partition, the acetonitrile layer was mixed with 600 ml of 2% sodium chloride aqueous solution. PB was extracted twice from this aqueous layer with 50 ml of n-hexane. The n-hexane layer that contained PB was dried by a small anhydrous sodium sulfate column. The elute was concentrated with a Kuderna-Danisch evaporative concentrator. The concentrate, to which was added 1 ml of 1000 ppm thymol in ethanol as an internal standard, was filled up to 5 ml with ethanol, and then applied to a high speed liquid chromatography for analysis.

High speed liquid chromatography A Hitachi Model 635 instrument with a fluorometric detector was used. An adsorption column of Hitachi Gel 3010 (2.1 I.D. X 500 mm) was employed with ethanol as a mobile phase (Flow rate: 1 ml/minute, Pressure:80 kg/cm^2, Temperature: room temperatures). A fluorometric detector was set with the excitation maximum at 290 nm and the emission maximum at 340 nm. The sample size of injection was 5 µl.

RESULTS AND DISCUSSION

Some procedures were reported to determine PB in agricultural products. Colorimetric determinations (SECREAST and CAIL 1971: AOAC 1975) were tedious and lacked selectivity to PB. Other compounds having dioxymethylene functional group were measured as PB. A gas chromatographic determination was reported (ISSHIKI and WATANABE 1976). It was still time-consuming because samples were cleaned up in twice by liquid column chromatography. The determination including the use of a high speed liquid chromatography with a fluorometric detector was accurate, rapid and simple (ISSHIKI et al. 1977). Owing to the above reason, a high speed liquid chromatography was applied in this investigation.

The typical high speed liquid chromatogram of PB is shown in Fig. 1. The relative retention time of PB to thymol was 2.50.

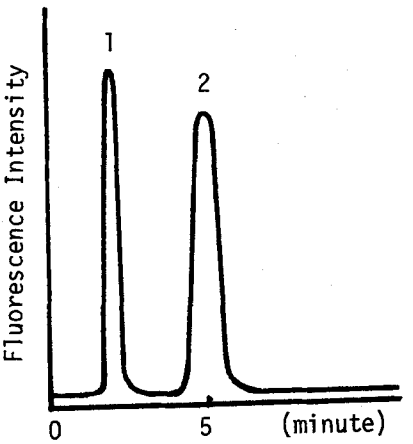

Fig. 1 Typical High Speed Liquid Chromatogram of Piperonyl Butoxide.
1, Thymol as an internal standard.
2, Piperonyl Butoxide.

TABLE 2

Concentration of Piperonyl Butoxide in Agricultural Products

Sample	Japan		U.S.A.		Canada		Australia		New Zealand		Indonesia		Thailand		Malaysia		Korea		Unknown	
	ppm	n*	ppm	n	ppm	n	ppm	n	ppm	n	ppm	n	ppm	n	ppm	n	ppm	n	ppm	n
Unhulled rice	ND	108	ND	8													ND	5		
Hulled rice	ND	90	ND	4													ND	6	ND	5
Wheat	ND	5	ND	13	ND	12	ND	5	ND	3	ND	5							ND	24
			0.2	2																
			1.4	1																
Barley	ND	5	ND	8	ND	6	ND	5	ND	2									ND	10
			0.8	1			0.3	1												
							1.4	1												
Buck wheat			ND	3	ND	8													ND	6
Rye			ND	3	ND	7	ND	8											ND	7
Oats			ND	4	ND	4	ND	11											ND	4
Corn	ND	8	ND	10	ND	2					ND	7	ND	10	ND	5			ND	14
Milo											ND	6	ND	8			ND	2	ND	4
Soy bean	ND	13	ND	12															ND	8
Red bean	ND	16																	ND	3

*The number of tested samples. ND: Not detected. The detection limit: 0.1 ppm.

PB added to each sample (0.4 ppm) was recovered in the range from 82.9 to 102%. In this experiment the detection limit was 0.1 ppm of PB in samples. It was superior to the detection limits of the colorimetric and gas chromatographic determinations.

The analyses of PB were carried out for 531 samples of 10 species. The results of the analyses were shown in Table 2. PB was detected from wheats and barleys harvested in U.S.A. and Australia. It was not detected from any other samples. Among 65 tested wheats there were 3 samples from which PB was detected. In case of 39 tested barleys, PB was also detected from 3 samples. The range of residual concentration of PB was from 0.2 to 1.4 ppm. WHO adopts 20 ppm of PB in raw cereals as tolerance limits. U.S.A. and Canada show the same limits as WHO. In Japan 24 ppm is admitted. The residual levels of PB in this investigation were much lower than these limits. PB was detected from only one percent of tested samples. (The detection limit of PB was 0.1 ppm.) It is not likely that PB would be used popularly to agricultural products.

PB shows effects on some drug metabolisms of mammalians. It was reported that the metabolism of 3,4-benzpyrene was inhibited by PB (FALK et al. 1965; EPSTEIN et al. 1967). The biochemical behavior and monitor of the residual levels of PB in our food and environment should be made clear as soon as possible.

SUMMARY

The residual levels of piperonyl butoxide (PB) in agricultural products were investigated for 531 samples of 10 species. For the determination of PB a high speed liquid chromatograph equipped with a fluorometric detector was used. The detection limit of PB was 0.1 ppm. PB was detected from 3 barleys and 3 wheats harvested in U.S.A. ans Australia. It was not detected from the others. The residual range was from 0.2 to 1.4 ppm.

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