Journal of Health Science, **56**(1) 88–91 (2010)

Efficiency of Rice Bran for Removal of Pretilachlor and Esprocarb in Artificial Gastric Fluid

Atsuko Adachi,[*, a] Yuki Okita,[a] and Jun Adachi[b]

[a]*Department of Hygienic Sciences, Kobe Pharmaceutical University, Motoyamakitamachi, 4–chome, Higashinada-ku, Kobe 658–8558, Japan and* [b]*Graduate School of Global Environmental Studies, Kyoto Uiversity, Yoshida-Honmachi, Sakyo-ku, Kyoto 606–8501, Japan*

(Received September 29, 2009; Accepted November 19, 2009; Published online November 20, 2009)

Rice bran was found to effectively adsorb pesticides in artificial gastric fluid. Equilibrium adsorption isotherms conformed to the Freundlich type (log-log linear). Pretilachlor and esprocarb were successfully removed in artificial gastric fluid with an average removal efficiency of 85.5% and 95.8%, respectively after 90 min when rice bran (10 g/l) was added to samples containing from 0.05 to 5 mg/l of pesticides. The removal of pesticides by rice bran was attributed to the uptake by intracellular particles called spherosomes.

Key words —— pretilachlor, esprocarb, spherosome, rice bran, Freundlich isotherm

INTRODUCTION

Pesticide residues in humans are mainly derived from the ingestion of contaminated food. Food is the main source of exposure of the general population to pesticides, and accounts for more than 90% of the total exposure.[1] Pesticide residues in foods and crops are a direct result of the application of pesticides to crops growing in field, and, to a lesser extent, from pesticide residues remaining in the soil.[2] There has been much interest in the use of organoclays as adsorbents to remove and remediate pesticide in contaminated agricultural soil.[3–5] Activated carbon, zeolite particles, kaolinite, fuller's earth and bentonite have been evaluated for the treatment of acute pesticide poisoning by oral ingestion.[6–9] Because of the hydrophilic character of their surfaces, clay minerals, particularly phyllosilicates, have been shown to be very good adsorbents for highly polar pesticides, but their adsorption capacity for organic compounds is usually low.[10–12] Our research has focused on the adsorption properties of rice bran.[13] The object of this work was to elucidate the effect of rice bran for removing pesticide in artificial gastric fluid.

MATERIALS AND METHODS

Apparatus —— The assay of pretilachlor or esprocarb was performed on a Shimadzu Model GC-14B gas chromatograph equipped with a flame ionization detector and a capillary column (ULBON HR-52, 30 m × 0.53 mm Shinwa Chemical ludustries, LTD., Kyoto, Japan). The column was maintained at 250°C, with both the injection port and detector were maintained at 280°C.

Materials —— Rice bran was purchased at a local market. Pretilachlor and esprocarb of analytical standard purity were purchased from Wako Pure Chemical Industries Ltd. (Amagasaki, Japan). Activated carbon (powder, coal based carbon) was purchased of practical grade from Wako Pure Chemical Industries Ltd.

Artificial Gastric Fluid —— Artificial gastric fluid was prepared by United States Pharmacopeia (USP) method.[14] Sodium chloride (2.0 g) and pepsin (3.2 g) were dissolved in hydrochloric acid (7.0 ml) and sufficient water to make 1000 ml. This solution has a pH of about 1.2.

Adsorption Experiment —— A 100 ml of sample solutions containing 0.05 or 5 mg/l of pretilachlor or esprocarb (Pesticides were dissolved in artificial gastric fluid) and rice bran (0.05–1.0 g) were placed into 100 ml glass stoppered Erlenmeyer flasks and mixed with a stirrer at 37°C. The reaction mixture was filtered through filter paper (quantitative ashless No. 5A Toyo Roshi, Ltd., Tokyo, Japan) to remove the rice bran. The initial 10 ml of filtrate was discarded because of the adsorption of pesticides by the filter paper. In control samples without rice bran, the subsequent filtrate after the discarded portion contained the same amount of adsorbent as the original solution. Fifty ml of this filtrate was

*To whom correspondence should be addressed: Department of Hygienic Sciences, Kobe Pharmaceutical University, Motoyamakitamachi, 4–chome, Higashinada-ku, Kobe 658–8558, Japan. Tel.: +81-78-441-7584; Fax:+ 81-78-441-7584; E-mail: a-adachi@kobepharma-u.ac.jp

placed in a separatory funnel and 5 ml of m-xylene was added to the solution. The mixture was shaken for 1 min. The separated m-xylene layer was subjected to gas chromatography (GC) to assay the concentration of pesticides. To assess the evaporation loss of pesticides, control experiments without rice bran were performed as above. Maximum loss was about 5% (4.7 ± 0.22%), although negligible loss was detected in most cases. The removal efficiency of rice bran was calculated after taking into account the evaporation loss of pesticides. Values are shown as means ± S.D.

Recovery Test—— To determine the method efficiency for pretilachlor and esprocarb, the recovery experiments were performed following the same procedure as for the sample treatment, except for the absence of rice bran.

Isolation of Spherosomes—— Spherosomes were isolated using an improved method based on that of Moreau *et al.*[15] Samples of 1 g (dry weight basis) of rice bran were ground in 40 ml of grinding medium consisting of 20 mM sodium succinate, pH 5.6, containing 10 mM $CaCl_2$ with a mortar and pestle. The paste was filtered through four layers of cheesecloth, and the filtrate centrifuged at 30000 × g for 20 min. The spherosome pad was removed from the surface with a spatula and washed by resuspending in 40 ml of fresh medium. This suspension was recentrifuged at 30000 × g for 20 min. This process was repeated two more times, and the final pellet was used as the spherosome fraction. The composition of rice bran and spherosome is shown in Table 1. Moisture content was determined by drying a sample for 6 hr at 110°C. Protein concentration was determined by the method of Kjeldahl.[16] Lipids were extracted by the Bligh and Dyer method.[17] The mass of the total lipid was determined by drying an aliquot of chloroform extract in a vacuum oven overnight and weighing the resulting lipid residue. Carbohydrate (glucide) was determined by Anthrone method.[18] Dietary fiber was determined by Association of Official Analytical Chemists (AOAC) method.[19]

Table 1. Composition of Rice Bran and Spherosomes

Constituent	Concentration (g/100 g)	
	Rice Bran	Spherosomes
Water	13.5	9.8
Protein	13.2	26.6
Lipid	18.3	3.9
Carbohydrate		
glucide	38.3	38.4
fiber	7.8	3.6
Ash	8.9	17.4

RESULTS AND DISCUSSION

Recovery Studies

The recovery of pretilachlor and esprocarb can be checked according to the procedures for the recovery test. The mean recoveries of added pretilachlor and esprocarb (0.05 or 5 mg/l) in the distilled water samples were 95.1–97.1% for pretilachlor and 95.8–98.1% for esprocarb. The limit of quantification was defined for GC as the sample concentration required to give a signal-to-noise ratio of 6 : 1. It was evaluated at 0. 001 mg/l of water.

Adsorption Rate

Table 2 shows efficiencies of rice bran for the removal of pretilachlor and esprocarb in artificial gastric fluid at a reaction time of 90 min, because the removal efficiency became constant after 60 min treatment. The average removal efficiencies for pretilachlor and esprocarb was 85.5% and 95.8%, respectively. This removal efficiency was similar to that of distilled water.

Adsorption Isotherm

The amount of pretilachlor and esprocarb adsorbed in the equilibrium state was plotted against

Table 2. Removal Efficiency of Rice Bran for Pretilachlor and Esprocarb in Artific Gastric Fluid

Substance	Concentration (mg/l)		Removal efficiency (%)
	Before treatment	After treatment	
Pretilachlor	0.05	0.005–0.007	87.2 ± 1.2[a]
	5	0.7 –0.9	83.7 ± 1.6[a]
Esprocarb	0.05	0.001–0.002	97.0 ± 0.2[a]
	5	0.2 –0.3	94.6 ± 0.9[a]

a) Data represent the mean ± S.D. of three separate determinations. Rice bran, 10 g/l; reaction time, 90 min.

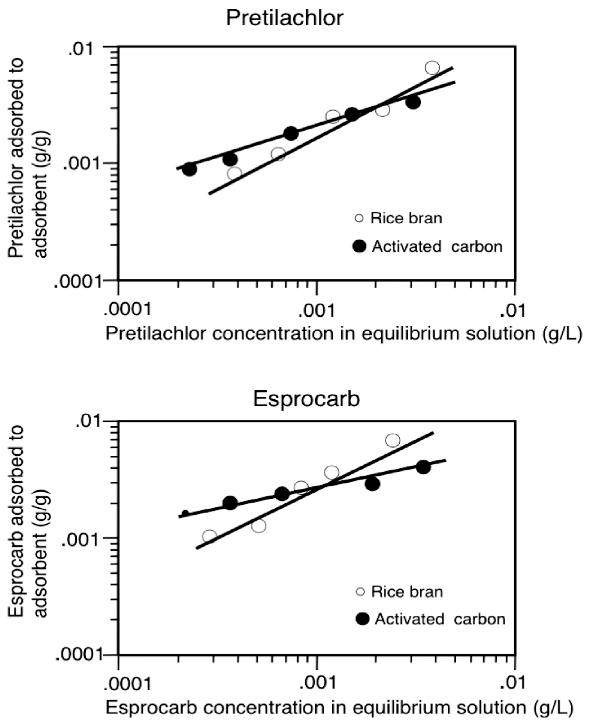

Fig. 1. Freundlich's Adsorption Isotherm of Pretilachlor and Esprocarb

Data represent the mean ± S.D. of three separate determinations. Reaction time: 3 hr, pretilachlor or esprocarb: 5 mg/l, pH: 1.2.

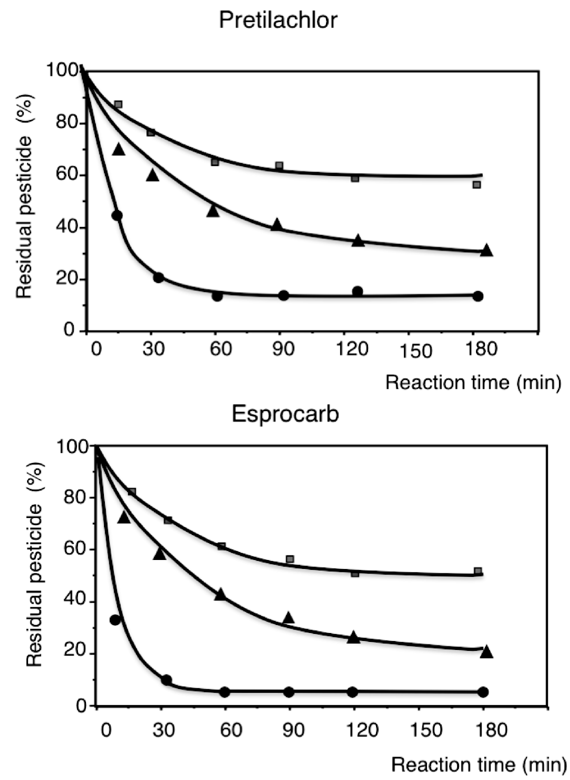

Fig. 2. Effect of Amount on of Rice Bran on the Adsorption of Pretilachlor and Esprocarb in Artificial Gastric Fluid

Data represent the mean ± S.D. of three separate determinations. Rice bran: (■) 1.0 g/l, (▲) 2.0 g/l, (●) 10 g/l, pretilachlor or esprocarb: 5 mg/l, pH: 1.2.

the concentration of these compounds in solution on a logarithmic scale. Equilibrium was measured after at least 3 hr of contact. A linear relationship was obtained, indicating that the adsorption reaction was of a Freundlich type (Fig. 1). This result indicated that the adsorption efficiency of rice bran for esprocarb and pretilachlor was higher than that of activated carbon in the high concentration range.

Effect of Amount of Rice Bran on Adsorption

Figure 2 shows the effect of the amount of rice bran on the removal of pretilachlor and esprocarb. The residual pesticide decreases in response to the amount of rice bran. The removal is initially fast, but after 60 min, the removal appears to plateau. In this experiment, 10 g/l of rice bran showed the highest efficiency.

Adsorption Mechanism

We investigated the mechanism of removal. We have previously reported that rice bran was effective in removal of organochlorine compounds such as chloroform, dichloromethane and benzene. Furthermore, it was confirmed that the spherosomes isolated from rice bran were effective in removing these organic compounds.[13] Analytical and laser microscopic data have confirmed that the removal of organochlorine compounds and benzene is dependent on the uptake of these compounds into intracellular particles called spherosomes.[13] Spherosomes are intracellular particles about 10 μm in diameter and widely distributed among plants and fungi.[20] Neither the function of spherosomes nor its analysis is well understood.

Spherosomes are organelles rich in lipid, and they differ in morphology and origin from large oil bodies.[21] Table 3 shows the removal efficiency of pretilachlor and esprocarb by spherosomes isolated from 1 g (dry weight basis) of rice bran. The removal by spherosomes was similar to that of rice bran. We regarded the special membranes to be related to the uptake of chemical compounds into spherosomes. The chemical nature of the spherosomes is uncertain. Based on the result, we concluded that removal by rice bran is dependent on the uptake into spherosomes.

Activated carbon has usually been used for oral use as detoxification.[6] At equilibrium, the adsorption efficiency of rice bran for pretilachlor and esprocarb was higher than that of activated carbon in

Table 3. Removal Efficiency of Spherosome Isolated from Rice Bran for Pretilachlor and Esprocarb in Artificial Gastric Fluid

Substance	Concentration (mg/l)		Removal efficiency (%)
	Before treatment	After treatment	
Pretilachlor	5	1.3–1.6	71.5 ± 3.8[a)]
Esprocarb	5	0.7–0.9	81.4 ± 4.6[a)]

a) Data represent the mean ± S.D. of three separate determinations. All spherosomes obtained from rice bran (1 g) were used for this experiment. Reaction time, 90 min.

the high concentration range. Our study showed that the use of rice bran as an adsorbent is an efficient method for the treatment of acute pesticide poisoning by oral ingestion. Rice bran is by product of making polished rice from brown rice. Therefore, rice bran is very inexpensive, costing 1/50 – 1/40 that of activated carbon. Additionally, the use of rice bran is significant from the aspect of effective utilization of waste matter.

Taken together, the findings of this study suggest that the use of rice bran as an adsorbent is an efficient and cost-effective method for removal of pretilachlor and esprocarb in artificial gastric fluid.

REFERENCES

1) Mills, P. A. (1936) Pesticide residue content. *J. Assoc. Off. Anal. Chem.*, **46**, 762–767.
2) Businelli, A., Vischetti, C. and Coletti, A. (1992) Validation of Koc approach for modelling the fate of some herbicides in italian soil. *Fresenius Environmental Bulletin*, **1**, 583–588.
3) Mortland, M. M., Shaobai, S. and Boyd, S. S. (1986) Clay-organic complexes as adsorbents for phenol and chlorophenols. *Clays Clay Miner.*, **34**, 581–585.
4) Boyd, S. A., Mortland, M. M. and Chiou, C. T. (1988) Sorption characteristics of organic compounds on hexadecyltrimethyl ammonium-smectite. *Soil Sci. Soc. Am. J.*, **52**, 652–657.
5) Hermosin, M. C. and Comejo, J. (1993) Binding mechanism of 2,4-dichlorophenoxy acetic acid by organoclays. *J. Environ. Qual.*, **22**, 325–331.
6) Hiraishi, M. (1987) The effect of oral adsorbent on surgically induced hepatic failure. *Surg. Today*, **17**, 517–527.
7) Walcarius, A. and Mouchotte, R. (2004) Efficient in vitro paraquat removal via irreversible immobilization zeolite particles. *Arch. Environ. Contam. Toxicol.*, **46**, 135–140.
8) Keizer, A. (1990) Adsorption of paraquat ions on clay minerals. Electrophoresis of clay particles. *Prog. Colloid Polym. Sci.*, **83**, 118–126.
9) Okonek, S., Setyadharma, H., Borchert, A. and Krienke, E. G. (2005) Activated charcoal is as effective as fuller's earth or bentonite in paraquat poisoning. *J. Mol. Med.*, **60**, 207–210.
10) Jaynes, W. F. and Vance, G. F. (1996) BTEX sorption by organo-clays:cosorptive enhancement and equivalence of interlayer complexes. *Soil Sci. Soc. Am. J.*, **60**, 1742–1749.
11) Celis, R., Koskinen, M. J., Hermosin, M. C., Ulibarri, M. A. and Cornejo, J. (2000) Triadimefon interactions with organoclays and organohydrotalcites. *Soil Sci. Soc. Am. J.*, **64**, 36–43.
12) Lee, J. F., Crum, J. and Boyd, S. A. (1989) Enhanced retention of contaminants by soils exchanged with organic cations. *Environ. Sci. Technol.*, **23**, 1365–1372.
13) Adachi, A., Ikeda, C., Takagi, S., Fukao, N., Yoshie, E. and Okano, T. (2001) Efficiency of rice bran for removal of organochlorine compounds and benzene from industrial wastewater. *J. Agric. Food Chem.*, **49**, 1309–1314.
14) US Pharmacopeial Convention, lne. (USA) (1995)
15) Moreau, R. A., Liu, K. F. and Huang, A. H. (1980) Spherosomes of castor bean endosperm. *Plant Physiol.*, **65**, 1176–1180.
16) Kjeldahl, J. (1883) Neue method zur bestimmung des stickstoffs in organischen korpern. *Ztschr Analytical Chemistry*, **33**, 366–382.
17) Bligh, E. G. and Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem.*, **37**, 911–915.
18) Scott, T. A. and Melvin, E. H. (1952) Determination of dextran with anthrone. *Anal. Chem.*, **25**, 1656–1661.
19) Southgate, D. A. T. (1969) Determination of carbohydrates in foods. *J. Sci. Food Agric.*, **20**, 331–335.
20) Buttrose, M. S. and Ikeda, C. (1963) Ultrastructure of the developing aleurone cells of wheat grains. *J. Biol. Sci.*, **16**, 768–774.
21) Jelsema, C. L., Morre, D. J., Ruddat, M. and Turner, C. (1977) Isolation and Characterization of the lipid reserve bodies, spherosomes, from aleurone layers of wheat. *Bot. Gaz.*, **138**, 138–149.