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《Original》

Behavior of ^{14}C-BHC Residues in Rice Grain

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米穀에 있어서 ^{14}C-BHC 殘留分의 行動

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Abstract

γ-(U-^{14}C)-BHC was applied to rice plants grown in a pot and its fate in the growth, polishing and oil-extraction processes of the grain was investigated.

The ^{14}C-activity was absorbed and translocated widely in the plant and the recovery of applied ^{14}C-activity in the straw and grain was about 2.8%, of which 9.4% was found in the brown rice. The % partitioning of ^{14}C-residues in bran and polished rice was 12:88 and that in oil and oilcake was 37:63. Characterization of ^{14}C-residues indicated the presence of γ-BHC, pentachlorocyclohexene, trichlorobenzene and hydrophilic metabolites, whose proportions were different in the straw and grain.

요 약

放射性 標識化合物인 γ-(U-^{14}C)-BHC를 폿트 재배한 水稻에 施用한 후 植物體에서의 分布 및 搗精, 搾油 과정중의 行方을 追跡하여 다음과 같은 결과를 얻었다.

^{14}C-放射能은 식물체에 吸收되어 各部位에 널리 分布되었고 볏짚과 穀粒에 移行된 放射能은 2.8%이었으며 그중 9.4%만이 玄米에 移行되었다. 玄米로 移行된 ^{14}C-放射能은 搗精과정에서 쌀겨 : 白米에 12:88의 비율로, 용매에 의한 搾油과정에서 기름 : 착유박에 37:63의 비율로 分配되었다.

水稻體로 移行된 ^{14}C-殘留分은 母體인 r-BHC와 代謝産物인 pentachlorocyclohexene, trichlorobenzene 및 親水性 分解産物임이 밝혀졌고 이들 成分의 比率은 볏짚과 玄米에서 각각 달리 나타났다.

1. Introduction

BHC (benzene hexachloride, also called HCH, hexachlorocyclohexane), one of the potent organochlorine insecticides, has been used in Korea since 1953 for crop and forest protection. Because of its persistent nature, great concern has been expressed in relation to accumulation in the environment and food chain[1,2].

Studies on the fate of BHC residues in rice grain were reported by several workers[3-9]. It is, however, deemed necessary

to study the magnitude and fate of BHC residues in the growth, polishing and oil-extraction of rice grain under local environmental conditions of Korea. This work employing radiotracer techniques was performed to confirm and extend the previous experiments conducted by means of conventional analytical methods.[10-12]

When this study was in progress, the domestic usage of BHC was banned effective July 1979 and its residue limit in cereal grains was set 0.1 ppm of total BHC in March 1981, by Korean Government (Ministry of Agriculture & Fisheries and Office of Environment, respectively).

2. Materials and Methods

1) Incorporation of ^{14}C-BHC to paddy rice plants

Paddy rice plants (an Indica type variety, Suwon 264) were transplanted on June 12 in plastic Wagner pots (24 cm diameter× 32 cm height) lined with double layers of polyethylene film to prevent leakage of water, under outdoor conditions. The pots were filled with paddy soils of sandy loam taken from the experimental farm of this Institute and flooded in 2-cm depth throughout the growing period by frequent irrigation. Eighteen of these pots were submerged side by side in a rectangular pit (2.2×1.1 m) so that the water surface of the pot was levelled with the surrounding water to simulate the practical field conditions. Fertilizers and pesticides other than BHC were applied according to conventional agricultural practices.

An exaggerated amount of γ-(U-^{14}C)-BHC (250 μCi, purchased from Radiochemical Centre Ltd., UK; 50 mCi/mmole in 5 ml toluene) was used for only one pot with 3 stumps of rice plants on August 25, the second generation time of rice stem borer. The labelled material was mixed with 0.6 g of 6% BHC powder (obtained from technical granular formulation made by the Korea Agricultural Chemicals Co. Ltd.) and 5 g of talc in a vessel made of aluminum foil. Twenty g of soil was thoroughly mixed with the above mixture and spread evenly on the soil surface after decanting the irrigated water. The pots were flooded again in 2 cm depth.

Three pots were left without application to serve as control, and 14 pots received treatment with cold BHC under conditions similar to those used for ^{14}C-BHC.

The plants were harvested on October 27, 63rd day after BHC application, by cutting 7 cm above the soil surface. Two plants treated with the labelled compound were taken for autoradiography, and the rest served for collection of the grains.

2) Treatment of rice grains

The harvested paddy grains were dehulled with hand and polished to 100% degree. Since the yield of brown rice obtained after ^{14}C-BHC application was not enough, 10 g of it was mixed with 140 g of brown rice after cold BHC application and subjected to polishing process. 5 g of rice bran thus obtained was extracted with n-hexane in a Soxhlet extractor for 24 hours and the solvent was evaporated under reduced pressure to obtain oil fraction.

3) Autoradiography

Rice plants were harvested and exposed to Fuji medical X-ray film by conventional methods[13] for 5 months in dark and developed by automatic developer of Sakura model QX 130.

4) Gas chromatography

BHC residues in rice samples were extracted and determined according to the previous report.[10]

5) Determination of ^{14}C-activity

Plant materials were digested and counted according to Mahin and Lofberg[14] as follows. Rice straw and grains were air-dried and powdered to 50 mesh. 0.1 g of solid samples was placed on the bottom of counting vials and 0.2 m*l* of 60% perchloric acid was added to each vial. When the samples were thoroughly wetted, 0.4 m*l* of 30% hydrogen peroxide was added. The vial caps were tightened and warmed to 75°C for 2 hours, with occasinnal agitation. After cooling to room temperature, 6 m*l* of Cellosolve and 10 m*l* of PPO solution (6 g PPO/*l* toluene) were added. The digested samples were counted in an Aloka LSC-601 Liquid Scintillation Counter; counting efficiency 88.3%. Extractives from plant materials were counted without digestion.

6) Preparation of BHC metabolites

BHC metabolites were obtained according to Nakazima et al.[15] as follows. 10 mg of γ-BHC (authentic sample from Polyscience Corp.) was dissolved in a mixture of 1.5 m*l* acetone and 2.5 m*l* N/50 NaOH contained in a 25 m*l* capped tube. The solution was kept 20 minutes at 38-39°C and then neutralized with N/10 HCl. The reaction mixture was extracted with 10 m*l* each of diethyl ether three times, and the combined extracts were dehydrated with anhydrous sodium sulfate and filtered. The filtrate was concentrated to dryness by air current and dissolved in 2 m*l* of n-hexane to serve as standard BHC metabolites in the thin-layer chromatography.

7) Thin-layer chromatography

The methods of Yule et al.[16] and Morley et al.[17] were adopted for TLC. Thin-layer plate made of silica gel G in 0.25 mm thickness on a glass plate was activated by heating one hour at 105°C and the sample after spotting followed by air drying was developed with 5% acetone in n-hexane (V/V) for n-hexane extracts and benzene-n-hexane (1:1, V/V) for methanol extracts[18] to travel about 10 cm distance. One developed plate was segmented into 1 cm zones from the origin, the silica gel from each zone was scrapped and counted for ^{14}C-activity in scintillation vials by adding scintillator solutions. Another developed plate was sprayed with coloring agent (1.7 g of silver nitrate was dissolved in 15 m*l* of 10% ammonium hydroxide and diluted to 200 m*l* volume) to locate the presence of metabolites. This developed plate was then passed through a radiochromatogram scanner (Technical Associates) to confirm the radioactive constituents. The operating conditions were; gate time 30 sec, chart speed 0.2 cm/min, and detector opening 1×5 mm rectangular.

3. Results and Discussion

1) Appearance of ^{14}C-residues in rice plants

Rice plants harvested on 64th day after application of γ-(U-^{14}C)-BHC were subjected to the autoradiography and measurement of radioactivity. The autoradiograph as shown in Fig. 1 indicated that the radioactivity was distributed in the stems, leaves and grains of the rice plant and was sufficient for activity counting.

A preliminary measurement of activity for a few plants gave the results as shown

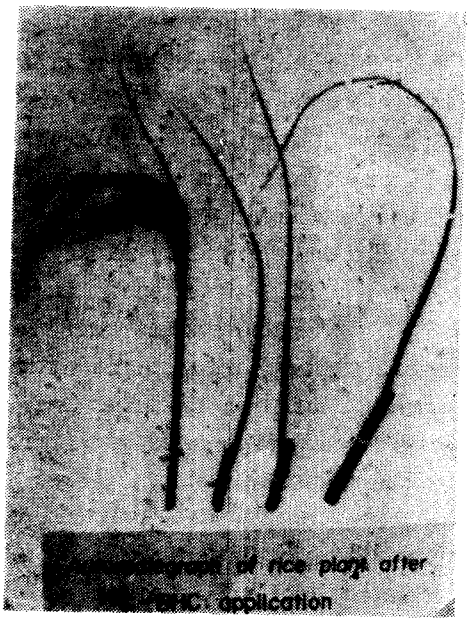


Fig. 1. Autoradiograph of Rice Plant after ^{14}C-BHC Application

in Table 1. The radioactivity of the plant material was enough for further experiment. It was also found that the crop after application of cold technical BHC showed a certain level of activity, which was, therefore, substracted from the activity of labelled samples in the forthcoming results.

The results obtained from a large amount of samples are given in Table 2. The % recovery of the applied ^{14}C-activity in the rice straw and grain was estimated to be about 2.8%. The level of ^{14}C-residues in the straw was 3 times higher than in the grain, which contained only 12.9% of the total recovered activity.

The gas chromatographic analysis of BHC residues in the brown rice samples after application of cold BHC showed the presence of 0.050 ppm α-BHC, 0.008 ppm β-BHC, 0.053 ppm γ-BHC, 0.004 ppm δ-BHC and 0.115 ppm total BHC. These levels were two times higher than those obtained in the field experiment.[10] The application rate of total BHC in the present pot

Table 1. A Preliminary Analysis of ^{14}C-residues in Rice Plants after ^{14}C-BHC Application

Sample	^{14}C-BHC applied		Cold BHC applied	
	cpm/0.1 g	μg BHC eq./g	cpm/0.1 g	μg BHC eq./g
Rice straw	9,807	7.50	76	0.052
Brown rice	3,235	2.47	7	0.005
Rice hull	1,820	1.39	96	0.077

*Data are means of triplicate analyses for samples from a few plants. ^{14}C-residues were calculated on the basis of 1.31×10^4 cpm/μg BHC equivalent.

Table 2. ^{14}C-residues in Rice Plants after ^{14}C-BHC Application*

Sample	Weight harvested (g)	Activity counted (cpm/0.1g)	Activity of controlsample (cpm/0.1g)**	True activity (cpm/0.1g)	^{14}C-residues*** (μg BHC eq/g)	% Recovery of ^{14}C-activity
Rice straw	58	20,693	227	20,466	15.64	2.42
Brown rice	21	6,337	155	6,182	4.71	0.26
Rice hull	8	6,165	289	5,876	4.48	0.10

*250 μCi of ^{14}C-γ-BHC with a specific activity of 6.67 μCi/mg γ-BHC was applied to one experimental pot having 3 stumps of paddy rice plants.

**Obtained by applying cold BHC instead of labelled pesticide in rice growing.

***Calculated on the basis of 88.3% counting efficiency and specific activity of initially applied BHC. (i.e., 1.31×10^4 cpm is equivalent to 1 μg of BHC).

Table 3. Partitioning of ^{14}C-residues in the Polishing Process of Brown Rice*

Sample	Weight (g)	Activity counted (cpm/0.1g)	Activity of control sample (cpm/0.1g)	True activity (cpm/0.1g)	^{14}C-residues (μg BHC eq/g)	% Partitioning of ^{14}C-residues
Brown rice	150	552	150	402	4.61	100
Polished rice	138	493	193	300	3.45	88
Rice bran	12	687	236	451	5.18	12

*10 g of the originally labelled brown rice was mixed with 140 g of cold brown rice for polishing and activity counting. But the ^{14}C-residues were expressed as if the whole sample was labelled, on the basis of 1.31×10^4 cpm/μg BHC equivalent.

experiment was calculated to be about 0.80 g/m^2 of surface soil whereas the rate in the field experiment was 0.19 g/m^2, equivalent to 3 kg of 6% granular formulation/10 a of rice paddy, the recommended level of application for the control of rice stem borer.

The level of ^{14}C-residues in plant materials as expressed by BHC equivalent from the specific activity of initially applied BHC was much higher than expected. This indicated that the ^{14}C-residues in plant materials should consist of parent and metabolic compounds of γ-BHC and that only 2.5% of total ^{14}C-residues was to be the parent BHC form in brown rice. This will be explained in connection with the characterization of ^{14}C-residues in the following experiments.

2) Partitioning of ^{14}C-residues in the polishing and oil-extraction processes

In polishing of brown rice, the bran fraction contained a little higher ^{14}C-residues than the polished rice grain, the partition ratio in terms of total activity in bran : grain being 12:88, as shown in Table 3. This is in contrast with the previous result obtained by gas chromatographic analysis in which BHC residues were highly concentrated in the bran fraction and the % partitioning of the residues in bran : grain were 92:8. It is most likely that the ^{14}C-residues in the polished grain largely consist of non-BHC hydrophilic metabolites originating from ^{14}C-BHC.

In oil-extraction process, oil fraction contained higher ^{14}C-residues than oilcake fraction or rice bran, the partition ratio in terms of total activity in oil : oilcake being 37:63, as shown in Table 4. This result also suggests that ^{14}C-residues in rice bran contain a large proportion of hydrophilic metabolites converted from ^{14}C-BHC.

3) Characterization of ^{14}C-BHC metabolites

In order to confirm the ^{14}C-BHC and its conversion products in rice plants, 1 g of rice straw and 3 g of brown rice to give the same level of radioactivity were extracted successively with n-hexane for 24 hours and with methanol for 48 hours in a Soxhlet extractor. The extracts were evaporated to dryness and after redissolving in a known volume of the same solvent, they were subjected to activity counting and thin-layer chromatography.

The calculated data on the partitioning of ^{14}C-residues in solvent extraction are given in Table 5. It was found that most of the ^{14}C-activity in rice straw was extracted by n-hexane while most of it in brown rice was neither extracted by n-hexane nor methanol, indicating that the constituents of ^{14}C-residues should be diff-

Table 4. Partitioning of ^{14}C-residues in the Oil-extraction Process of Rice Bran*

Sample	Weight (g)	Activity counted (cpm/0.1g)	Activity of control sample (cpm/0.1g)	True activity (cpm/0.1g)	^{14}C-residues* (μg BHC eq/g)	% Partitioning of ^{14}C-residues
Rice bran	5.0	687	236	451	5.18	100
Oil	1.2	977	194	783	8.99	37
Oilcake	3.8	635	223	412	4.71	63

*Rice bran obtained from diluted brown rice was used for oil-extraction and activity counting. But the ^{14}C-residues were calculated as if the whole sample was labelled, on the basis of 1.31×10^4cpm/μg BHC equivalent.

Table 5. Partitioning of ^{14}C-residues in Solvent Extraction of Rice Plants*

Fraction	Rice straw	Brown rice
n-hexane extract	90%	6%
Methanol extract (after hexane)	8%	8%
Residue after solvent extractions	2%	86%

*Radioactivity was measured for aliquots of each extract and expressed in terms of % partitioning of total activity after subtracting activity due to control samples.

erent in the different part of the crop.

Thin-layer chromatogram and radioactivity of the spots obtained from n-hexane extracts are shown in Fig. 2 and 3. Three spots including parent γ-BHC, pentachlorocyclohexene and trichlorobenzene were detected in the chromatogram. Brown rice showed a high activity at the location of

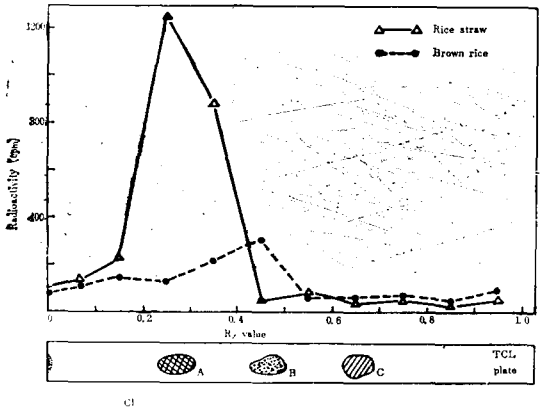


Fig. 2. Thin-layer Chromatogram and Manual Radioscan of n-hexane Extracts from Rice Plants Authentic compounds:
A, γ-BHC
B, pentachlorocyclohexene
C. trichlorobenzene

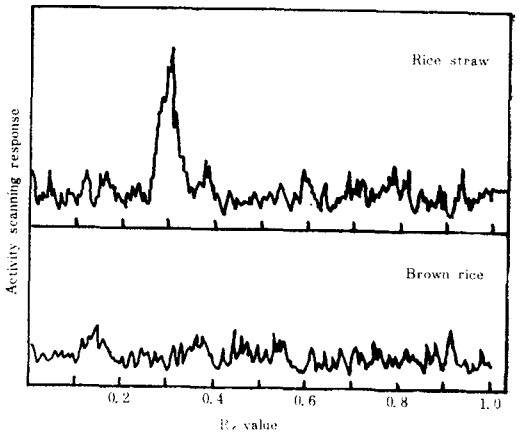


Fig. 3. Automatic Radioscan of Thin-layer Chromatogram of n-Hexane Extracts from Rice Plants

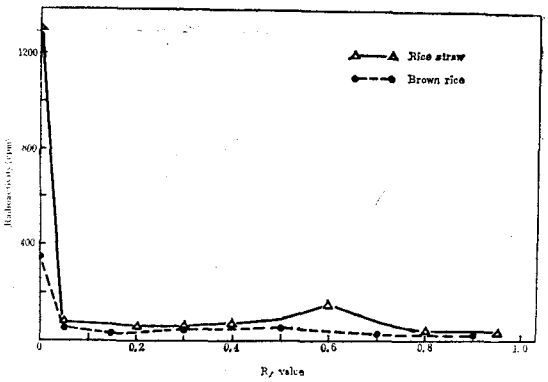
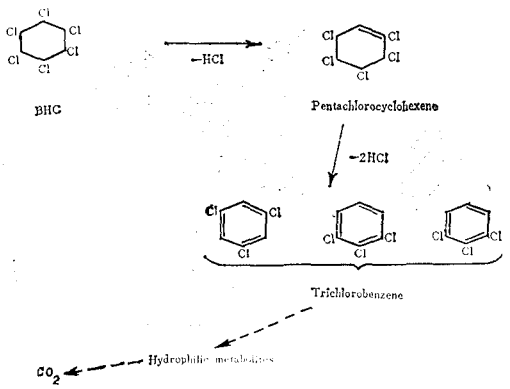


Fig. 4. Manual Radioscan of Thin-layer Chromatogram of Methanol Extracts from Rice Plants

pentachlorocyclohexene while rice straw showed a very high activity at the position of γ-BHC. Thin-layer chromatograms of methanol extracts showed high activities at the origin in both samples, indicating the presence of polar metabolites, as shown in Fig. 4.

The presence of high radioactivity in the extraction residue of brown rice may indicate the occurrence of non-BHC hydrophilic metabolites converted from ^{14}C-BHC, which might have been translocated from soil or plant tissues into the grain at the later stage of maturation. It is also interesting to note that BHC metabolites in the grain are different from those in the straw. Moreover, it should be pointed out that most of the ^{14}C-activity in brown rice was non-BHC hydrophilic metabolites and the apparently high ^{14}C-residues observed in brown rice might be explained from this aspect. This also suggests the possibility of misinterpreting experimental results obtained by radiotracer techniques in biological materials unless the constituents of labelled metabolites are clarified.

The metabolism of BHC isomers has been studied in relation to insect resistance and mammalian toxicology and more recently in relation to the persistence in the environment[2]. Information in plants or microorganisms appears to be fairly limited. However, the following metabolic pathway of BHC in rice plants and paddy soils can be easily assumed from earlier reports and this study.



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