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Determination Method for Residual 1,3-Dichloro-and 1,3,5-Trichloro-2-(4-nitrophenoxy) benzene (NIP and CNP) in Fish and Shellfish

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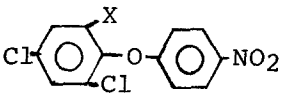
In the previous paper, the authors reported the contamination of a species of shellfish, short-necked clam, by a considerable amount of 1,3,5-trichloro-2-(4-nitrophenoxy)benzene (CNP), a herbicide widely used in Japan (YAMAGISHI et al. 1978). Annual production of CNP and its close analogue, 1,3-dichloro-2-(4-nitrophenoxy)benzene (NIP), reached 5000 and 300 tons, respectively in 1976 in Japan, and both compounds have been extensively applied especially to paddy rice field. However, little is known on the levels of environmental pollution by these compounds.



X=Cl CNP
X=H NIP

Figure 1. Structure of CNP and NIP.

There are several reports on the determination of CNP, NIP, and their metabolites from vegetables, cereals, and soil samples (ADLER and WARGO 1975, GOTOH 1967, KVALVAG 1974, TATSUKAWA et al. 1973, TOHYAMA and TAMAGAWA 1976, YAMADA 1975, YAMADA and AKUTSU 1974). However, the methods described in these reports have some shortcomings, when applied on fish or shellfish, because these samples are often contaminated by other organochlorine compounds such as PCBs and DDTs.

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The electron capture gas chromatography technique proposed in the present report principally follows that of the one for PCBs. Namely, the method involves homogenization of specimens, extraction by solvents, clean-up through saponification and chromatography on a Florisil column before gas chromatography. DDTs can be eliminated effectively by the saponification and PCBs by the Florisil chromatography.

MATERIALS AND METHODS

Reagents and Apparatus

CNP: separated from a commercial product, m.p. 107°C.

NIP: recrystalized from ethanol, m.p. 72°C.

PCBs: Kanechlor KC-400, and KC-500.

Each running standards of CNP and NIP are 0.02 ppm in n-hexane. PCBs are 1 ppm of a mixture of KC-400 and 500 (1:1) in n-hexane.

Florisil: PR grade, 60-100 mesh, was activated at 650°C for 18 hr and stored in a dessicator.

Other reagents and solvents were of pesticide or spectroscopic grade.

High speed blender: Ultra Turrax TP- 18/2N.

Gas chromatography: Shimadzu GC-5AP3FE, equipped with a ^{63}Ni electron capture (ECD) and dual flame ionization detector (FID). A digital integrator, Shimadzu ITG-2A was used.

Operating conditions:
Columns; column 1, OV-1 (2% on Gaschrom Q, 80/100 mesh) N_2 47 ml/ min, column 2, OV-17 (2% on Chromosorb W, AW DMCS, 80/100 mesh), N_2 80 ml/ min, column sizes 3 mm × 2 m glass columns. column temperatures 220°C, injector and detector temperatures 280°C.

Proposed Procedure

Figure 2 illustrates an outline of the proposed analytical procedure for CNP and NIP in fish and shellfish. About 50g of a sample is homogenized with a high speed blender, and 10g of it is weighed accurately. It is placed in a 300 ml Erlenmeyer flask, mixed and shaken with 10 ml of methanol and 200 ml of hexane, and then allowed to stand overnight at room temperature.

After the filtration with a glass filter, the residue is extracted again with 10 ml of methanol and 50 ml of hexane. The combined filtrate is shaken twice with 30 ml portions of water, dried over anhydrous sodium sulfate (30g). The sodium sulfate is rinsed with 30 ml of hexane, and the combined solution is concentrated to almost dryness at 40°C by the use of a Snyder column. The concentration procedures described in this methos are always carried out as this.

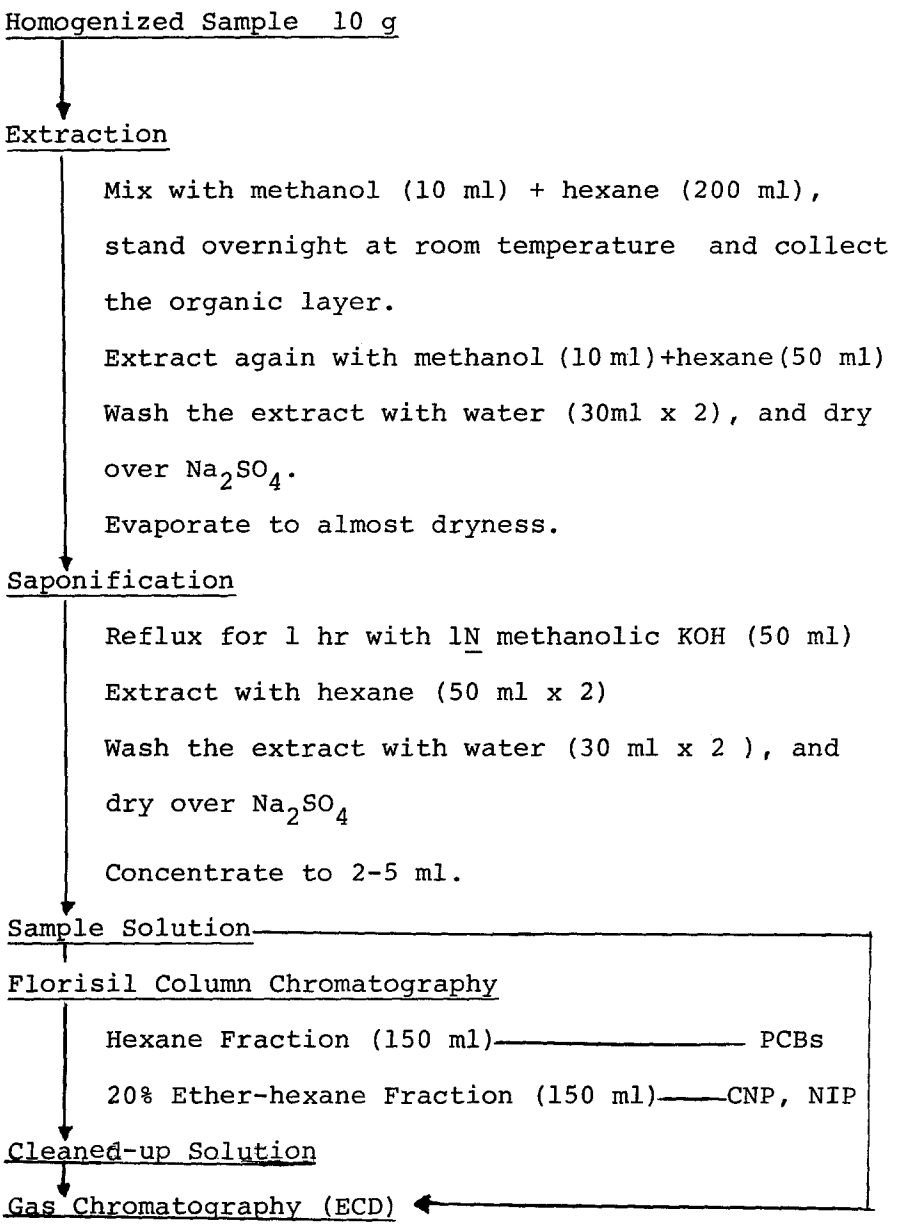

Figure 2. Scheme of CNP and NIP Analysis in Fish and Shellfish

The extract is refluxed for one hour with 50 ml of 1 N methanolic potassium hydroxide. After the solution is cooled below 50°C, it is extracted twice with 50 ml portions of hexane. The combined hexane is washed twice with 30 ml portions of water, dried and concentrated to 2 ml.

The solution is analyzed by ECD gas chromatography with or without further clean-up.

For further clean-up and the separation of CNP and NIP from PCBs, the concentrate is chromatographed on a Florisil column (2.2 × 10 cm) in hexane. The concentrate is tranfered to the column with three 1 ml portions of hexane. PCBs are eluted with 150 ml of hexane. Next, CNP and NIP are eluted with 150 ml of an ether-hexane mixture (20:80). These fractions are concentrated to 1-5 ml, and analyzed by ECD gas chromatography.

An aliquot (1-5 l) of the sample and standard solution is injected onto column 2.

RESULTS AND DISCUSSION

The Clean-up with Sulfuric Acid

Treatment with concentrated sulfuric acid has been used in the residue analyses of NIP (KVALVAG 1974) or PCBs (AKIYAMA et al. 1975). To check its applicability to pre-clean-up, the partition values of CNP and NIP were measured between hexane and concentrated sulfuric acid (97.2%); 1ml of n-hexane solution containing 1 mg each of CNP, NIP and 5α-androstane, the internal standard, was shaken vigorously with 0.3 ml of the acid. 54 percent of CNP and 33 percent of NIP remained in the hexane layer, based on the assumption that 5α-androstane resided quantitatively in the hexane layer. Because the volume of the hexane layer decreased, when shaken with the acid, the apparent values were somewhat higher; 68 and 42, respectively. With practical CNP level of a sample (2ppm in hexane), recovery was 71% and this agreed with the result of above experiment which performed in higher concentration level. With the diluted acid the recoveries increased, but it resulted in insufficient clean-up. The solubilities of organic compounds in sulfuric acid depend on the following mechanism; protonation, physical dissolution, sulfonation and so on (NENITZESCU 1968). Proton-donating abilities of strong acids are generally expressed with Hammett's H_0 function, which changes considerably in highly concentrated region (GOULD 1960). Therefore, partition values of both herbicides and interfering substances may vary in practical samples which contain water and other proton-acceptors to various extent. This may cause an error especially in analyzing wet samples. With this reason we did not adopt this treatment for pre-clean-up procedure.

Clean-up with Saponification

CNP and NIP were found persistent during the saponification. A slight modification of the saponification procedure for PCBs analysis recommended by the ADVISORY COMMITTEE of the MINISTRY of HEALTH and WELFARE (1972) was effective enough to remove interfering peaks in gas chromatography.

When the residue levels of CNP and NIP were sufficiently high concentration (above 20 and 15 ppb respectively), no more purification was necessary before gas chromatography.

Column Chromatography

Five milliliters of n-hexane solution containing 0.1 µg each of CNP, NIP and 5 µg each of PCB mixture, KC-400 and 500 were applied to a Florisil column and the eluate was examined. PCBs were eluted in the first fraction (150 ml of hexane) with recoveries of 98.8 and 101.3% in duplicated runs. CNP and NIP were recovered with 97.1, 100.4 and 97.3, 102.5%,respectively.

Silica gel (Wako, S-1) was similarly activated, packed and worked up exactly according to the procedure. PCBs were recovered in hexane with 97.8 and 98.6%. CNP and NIP in the ether-hexane (20:80) fraction resulted in 77.0, 79.8 and 86.3, 89.6%, respectively. Although, there may be more favourable conditions for silica gel, Florisil column chromatography seemed better.

Gas Chromatography

Column packings were examined to get good separation from organochlorine pesticides (Figure 3). On the OV-17 column, CNP and NIP peaks were found to overlap on p,p'- and o,p'-DDTs,repectively. However, the interference was removed by saponification, which dehydrochlorinated DDTs into the corresponding DDEs. On the OV-1 column, CNP was separated from p,p'-DDT, but NIP was difficult to be free from interference with p,p'-DDD. High level of PCBs interfered on all examined columns including 1.5% QF-1, 2% OV-225,and a mixed column, 10% DC-200 + 15% QF-1. OV-17 seemed the best column among them. Florisil chromatography was necessary in such a case.

When the column was conditioned at higher temperature (above 240°C) for a long time (72 hr), CNP peak decreased and split into two components, and after thirty times of repeated injections, this accompanying peak disappeared. Degradation or isomerization might occured on the active site of silicate support. Aging at lower temperature is recommended for the conditioning.

Recovery from Practical Samples

Recovery testswere carried out by spiking the samples with the level of 20 µg/kg each of CNP and NIP.

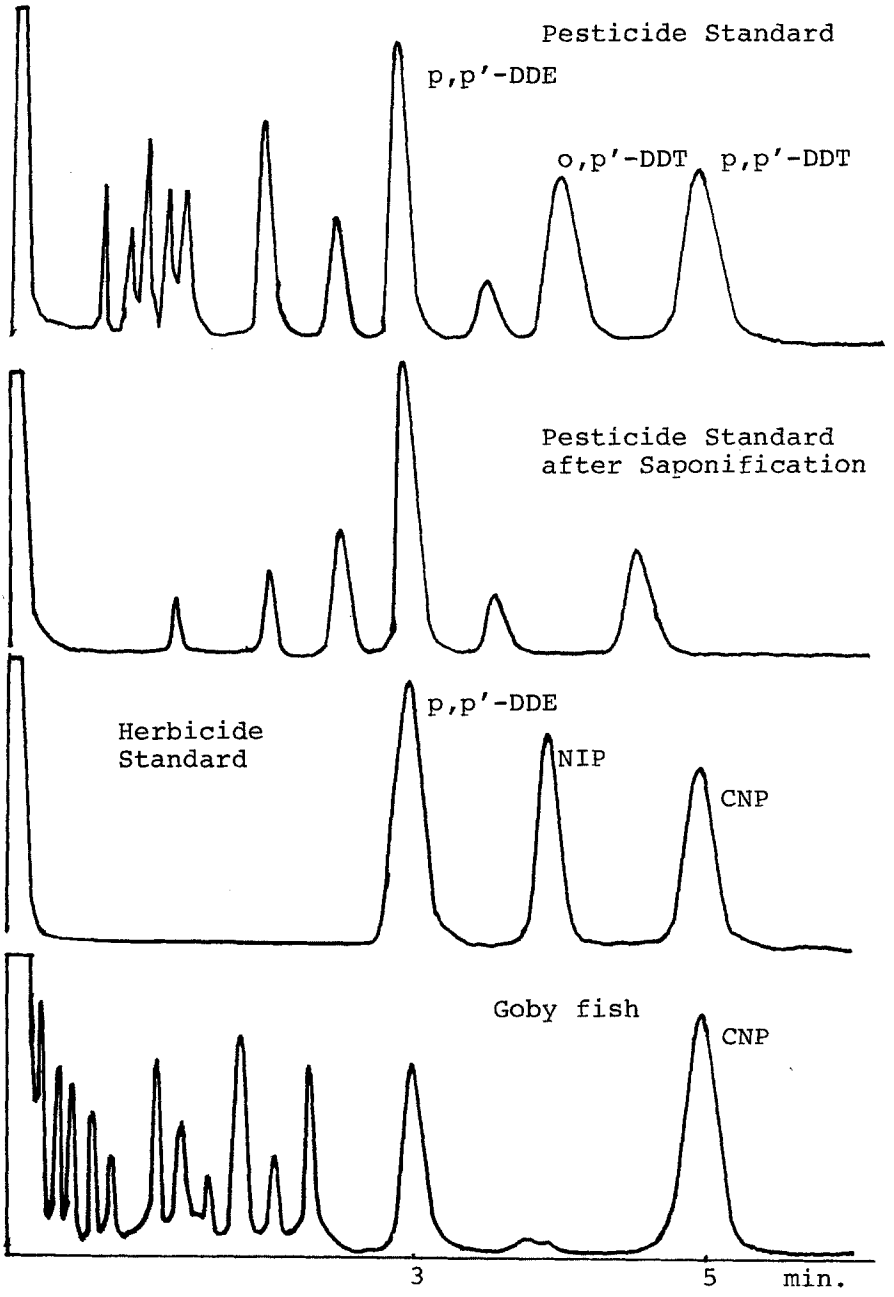

Figure 3. ECD Gas Chromatogram of Pesticides,Herbicides and Fish Sample. (2% OV-17, 2 m, 220° C)

The samples were fish (*Acanthogobius flavimanus*) and short-necked clam (*Tapos japonica*) collected in Tokyo Bay. Recoveries and standard errors of PCBs, CNP and NIP were 96.5 ± 2.1, 97.3 ± 2.3, and 93.2 ± 3.3% (average ± standard error), respectively, in triplicated runs, and no interfering peaks were found in the extract. Sensitivities of this method were 0.5 and 0.3 ppb for CNP and NIP, respectively, based on a 10g sample.

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