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Determination of Polynuclear Aromatic Hydrocarbons Contaminated with Chlorinated Hydrocarbon Pesticides

Takashi Negishi

Department of Agro-Environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

Polynuclear aromatic hydrocarbons (PAH) as a pollutant is well established in the environment (GRIMMER and BOHNKE 1975, PIERCE and KATZ 1975). For determination of PAH (benzo(a)pyrene), spectrofluorophotometry (MOORE et al. 1967, DUNN 1976) and gas chromatograph-mass spectrometry (GC/MS, LAO et al. 1973) have been developed recentry. Further, chlorinated hydrocarbon pesticides (pesticides) have been analyzed by the official method using gas chromatography equipped with electron capture detector (AOAC, SMART et al. 1974).

In our preliminary experiment (NEGISHI 1976), PAH showed almost the same character to pesticides in the preparatory steps, usually acompanying each other. HOWARD et al. (1966) analyzed PAH in smoked foods with alkaline digestion treatment, however they did not describe in detail the relation to pesticides. We investigated the effect of this treatment with pesticide contaminated PAH and the results showed the disappearance of all the added pesticides except PAH. This communication describes sensitive and reliable method which can be used to measure not only benzo-(a)pyrene but also other PAH in bovine adipose tissue with GC/MS tequnique.

An analytical grade of chemicals, anthracene and chrycene(Tokyo Kasei Co.), fluoranthene (Eastman Kodak Co.), benzo(a)pyrene, dibenzo(a,h)anthracene, phenanthrene, pyrene, aldrin, α-, β- and γ- BHC, DDD, DDT, Dieldrin and endrin (Wako Chemical Co.) were purchased commercially. Solvents were pesticide analysis grade (Kanto Chemical Co.). Potassium hydroxide was reagent grade and was used without further purification. Wako gel B-5 (Wako Chemical Co.) was used for thin-layer chromatography. The adipose tissue was obtained from commercial source.

An aliquate amount of the mixture of standard PAH and pesticides were added to 30 ml of hexane and partitioned with the same volume of acetonitrile saturated with hexane. The acetonitrile layer was dried under vacuo and treated with IN KOH in ethanol for 1 hr at 100°C. The extracts with hexane of the alkaline digest were concentrated to a fixed volume. The separation and qualitative identification of PAH and pesticides were performed by thin-layer chromatography using chromatoplates coated with Wako gel by

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the procedure of KUNTE (1967). The PAH developed with hexane-benzene (95:5) was detected by ultraviolet lamp (Manasuru Chemical Co., 2536 Å). The adsorbant at the position of standard PAH was scraped off the plate and transfered into a column (0.5 X 20 cm). The eluates with benzene (PAH) were used for further analysis.

The GC/MS used was Hitachi Model RMU-6MGC. A glass column (0.3 X 200 cm) packed with 2 % Dexsil-300 on 60/80 mesh chromosorb W was employed for analysis of both pollutants. Temperature was programmed from 160 to 290° at 3°/min. Carrier gas flow rate was 40 ml/min helium. Analysis of the tissue was carried out with the same method by use of 20 g of the tissue.

The thin-layer chromatogram of the standard mixture of PAH and pesticides and the extracts of alkaline digest are shown in Fig. 1. The PAH added revealed an almost intact form (no decomposed spot was detected), while the pesticides did not appear at all. It was suggested that the use of alkaline digestion treatment removed interference of pesticides in the PAH analysis.

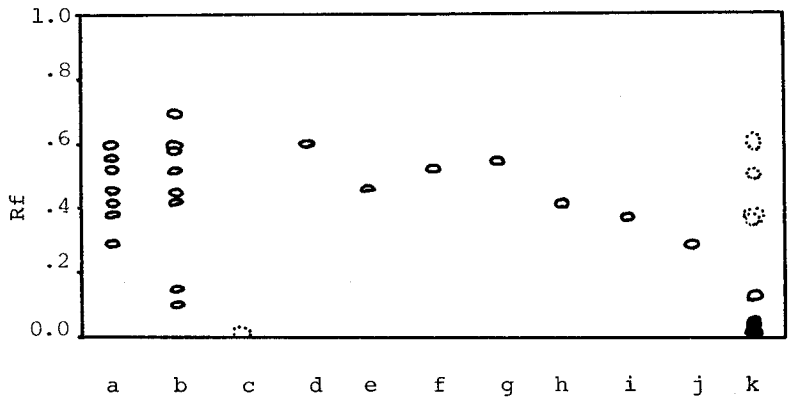

Fig. 1. Thin-layer chromatogram of the PAH and pesticides

a = mixture of PAH b = mixture of pesticides c = alkaline digest of pesticides d = anthracene e = phenanthrene f = fluoranthene g = pyrene h = chrycene i = benzo(a)pyrene j = dibenzo(a,h)anthracene k = extracts of the tissue

The recoveries of the PAH added to the tissue with GC/MS are shown in Table 1. The values were found between 80 to 97 %, and were resonably sufficient as compared to the references (HOWARD et al. 1966, DUNN 1976), but in this method, separation of anthracene and phenanthrene was unsuccessful.

The distribution of PAH identified in the bovine adipose tissue is shown in Table 2. Four main and 4 minor components were detected.

TABLE 1
Recoveries of PAH added to 20 g of bovine adipose tissue

PAH	Recoveries
	%
Anthracene + Phenanthrene	88.5*, 80.8*
Fluoranthene	97.6 , 95.0
Pyrene	86.4 , 90.5
Chrycene	89.5 , 87.4
Benzo(a)pyrene	85.4 , 87.4
Dibenzo(a,h)-anthracene	86.8 , 80.0

* Calculated from peak area X 1/2 (anthracene and phenanthrene contained the same weight)

TABLE 2
Tentative identification of PAH in bovine adipose tissue

Relative retention time	Mass number	Content	Tentative identification
		ppb	
0.382	210	46.0	dihydrotrimethylfluorene
0.461	178	1.0	anthracene and phenenthrene
0.843	204	33.5	dihydrofluoranthene
1.000	202	1.0	fluoranthene
1.098	202	30.0	pyrene
1.637	228	-	chrycene*
1.667	278	9.9	octahydrotrimethyl-benzoanthracene
1.902	256	17.2	tetrahydrobenzopyrene
2.186	252	1.0	benzo(a)pyrene
2.569	278	-	dibenzo(a,h)anthracene*

* standard

The identification of PAH was based on the relative retention time and its mass number(HOWARD et al. 1966) and the calculation of content were expressed in the foot note of Table 1.

The results indicate that the pollution by PAH is present in animals and metabolizes to hydro- and methyl- derivatives, when PAH passes through the liver. This evidence will be expressed in the next report.

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