Bull. Environm. Contam. Toxicol. 26, 424-427 (1981)

Tissue Distribution of Hexachlorophene in Lactating Cows

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Hexachlorophene(HCP) is an anti-microbial agent and is used as the vermicide against liver flukes(Fasciola hepatica)in cattle(DORSAM 1959), although HCP should be considered toxic to animals(KIMMELL et al. 1972, KENNEDY et al. 1975). KENNEDY et al.(1977) reported recently that HCP was detected in milk obtained from HCP-treated lactating rats. Therefore, it is important to study the accumulation of HCP in various tissues and milk of lactating cow.

This study was undertaken to determine the amounts of HCP and copper in tissues and milk from lactating cow when the cow received the mixture of HCP and cupric glycinate as vermicide.

MATERIALS AND METHODS

Two lactating cows, weighing ∿550 kg, received orally the mixture of HCP(4 mg/kg) and cupric glycinate(10 mg/kg)with diet once a day for 2 days and killed at 3 days after the last dosing. Livers, kidneys, muscles, adipose tissues and blood were isolated and chilled immediately. A control cow was killed at the same time. Milk was collected from four animals at 0, 6 and 24 h after the first dosing and at 6, 24 and 48 h after the second dosing.

One mL of milk was diluted with 1 mL of water and the mixture was acidified by the addition of diluted HCl. HCP was extracted with 10 mL of chloroform-methanol(1:1, v/v). The chloroform phase was collected. The extraction was repeated with 5 mL of chloroform. Adipose tissue was homogenized with 10 volumes of chloroform-methanol(1:1, v/v)by using a Potter glass homogenizer and treated in the same manner as described above. Other tissues were homogenized with 4 volumes of water and then acidfied with diluted HCl. HCP in 1 mL of the homogenate was extracted with 10 mL of chloroform-methanol(2:1, v/v) following the extraction with 5 mL of chloroform. HCP in the combined extract was back-extracted with 10 mL of 0.2 N NaOH and the aqueous phase was then extracted with chloroform. The recovery of HCP from milk and tissues was almost quantitative. HCP in blood was extracted with

0007-4861/81/0026-0424 $01.00

ether-ethanol(18:7, v/v)as described by ULSAMER(1972). After the acetylation with acetic anhydride-pyridine (1:1, v/v), some interfering materials were removed with silica gel G according to the method reported by ULSAMER (1972).

Detection of HCP was by gas chromatography with ^{63}Ni detector. A 2.6 mm i.d. x 1.7 m glass column packed with 3 % OV 101 on 80-100 mesh Gas-chrom Q was used. The column was pretreated with 5 % dimethyldichlorosilane in toluene and flushed with methanol. Nitrogen was used as the carrier gas at a pressure of 1.5 kg/cm^2. Column and detector temperatures were 245 and 270°C, respectively. Peak area of gas chromatogram was measured by triangulation and compared with standard curves prepared from standard HCP.

Concentration of Cu in all samples were determined by atomic absorption spectometer after dry ashing followed by wet ashing in nitric and sulfuric acids.

RESULTS AND DISCUSSION

Table I shows the concentration of HCP in some tissues of lactating cows treated with HCP and cupric glycinate. Livers showed the highest HCP concentration of 210-230 ng/g wet tissue. The concentration in other

TABLE 1 Tissue distribution of HCP in lactating cow

Tissue	Cow 1	Cow 2
Liver	210	230
Kidney	tr[a)]	tr
Muscle	35	20
Adipose tissue	5	10
Bolld	10	6

Values expressed as ng/g wet weight of tissue or ng/mL of fluid.
a) less than 5 ng/ wet weight of tissue.
HCP was not detectable in control.

tissue decreased in the order of muscle>adiopse tissue≈ blood and the amounts of HCP in kidney was negligibly low. The trend of accumulation of HCP in liver was reported in other species(CHOW et al. 1978, MILLER et al. 1978). The relative distribution of HCP in various other tissues, however, seems to be changed by experi-

mental conditions and species of animal used. At present we don't have any evidence to elucidate the extremely low accumulation of HCP in lactating cow kidney compared to relatively high accumulation of HCP in rat or guinea-pig kidney reported by CHOW et al. (1978).

Table 2 presents the concentration of HCP in milk. No HCP was detected at 6 h posttreatment, but there were detectable amounts of HCP in some milk sample at 24 h after the administration of HCP and cupric glycinate. Furthermore, it is not always likely that additional administration of HCP and cupric glycinate mixture at 24 h after the first administration would increase the HCP concentration in milk. By and large, the concentration of HCP in milk was extremely low compared to other tissue listed in Table 1, except for kidney. According to KENNEDY et al.(1977), oral administration of HCP(10 mg/kg)to lactating rats resulted in maximum concentration (∿1 ppm)in milk at 8 h after the treatment. Therefore, the concentration of HCP observed in milk of lactating cow is lower compared to that of lactating rat reported by KENNEDY et al.(1977).

TABLE 2 Concentration of HCP in milk

Animals	1	2	3	4
Predosing	0	0	0	0
6 h after first dosing	nd[a)]	nd	nd	nd
24 h "	8	tr[b)]	nd	nd
6 h after second dosing	nd	nd	nd	nd
24 h "	8	8	nd	tr
48 h "	nd	nd	nd	nd

Values expressed as ng/mL of fluid.
a) not detectable(less than 1 ng/mL).
b) less than 5 ng/mL

We determined the Cu concentration in liver, muscle, kidney, blood and milk after the administration of HCP and cupric glycinate. However, no significant change of the concentration from controls was found(data not given).

ACKNOWLEDGEMENT: We thank S. Nakagawa for technical assistance.

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