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Accumulation and Elimination in Mouse Fat of Compounds from the Mothproofing Agent Eulan WA Neu

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Two polychlorinated aminodiphenyl ethers were found in fish caught downstream of textile factories (Westöö and Norén 1977; Wells 1979). They were identified as 2',3,4,4',5-pentachloro-2-aminodiphenyl ether (I) and 2',3,4,4',5,6-hexachloro-2-aminodiphenyl ether (II) (Westöö and Norén 1977, Westöö et al. 1983), Figure 1. These compounds emanated from the mothproofing agent Eulan WA Neu (Farbenfabriken Bayer AG). The main active constituents of this agent are the N-chloromethylsulphone derivatives III and IV (Figure 1) of the compounds I and II. Human exposure to these substances can occur in textile industries or via ingestion of contaminated fish. Milk from two mothers who had worked with Eulan WA Neu did not contain I or II (detection limit 0.1 µg/kg). However, these mothers had already nursed their infants during several months before the milk was collected and the elimination rate of these substances is not known. To gain further information, the accumulation and elimination of I and II were studied in mice given compounds I-IV in the feed.

MATERIALS AND METHODS

Compounds I and II were prepared according to Westöö and Norén (1977). Pure I or II, or mixtures of the two were given to mice.

When the mixture of the active ingredients (mainly III and IV) of Eulan WA Neu was recrystallized from ethanol, IV was concentrated in the crystals and III in the solution. After several recrystallizations, III and IV were isolated from the purest fractions by column chromatography according to Wells (1979). The purified compounds were used as standards for the gas chromatographic determination of the concentrations of III and IV in the mixtures of III and IV or their sodium salts fed to the mice.

The animals were white, adult, female mice (strain NMRI) weighing about 25 g. Known amounts of compounds I and/or II, or III and IV in ethanol solution were applied to the feed, which was then dried at room temperature. When a mouse had consumed all

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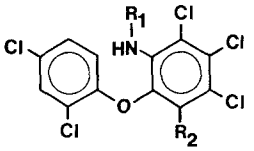

I R_1=H; R_2=H
II R_1=H; R_2=Cl
III R_1=SO_2CH_2Cl; R_2=H
IV R_1=SO_2CH_2Cl; R_2=Cl

Figure 1. Structures of the compounds discussed.

of the daily dose, uncontaminated feed was provided ad libitum. Thus the mice received defined daily doses. The animals were kept in individual cages. Control animals were fed uncontaminated feed parallel with each experiment. The abdominal fat was analyzed for I and II.

To study the times required from the consumption of I and II to their incorporation into the abdominal fat, five mice were given daily doses of 82.5 µg I and 190 µg II for 16 days and subsequently uncontaminated feed. From each mouse one fourth of the abdominal fat was removed on the 1st, 3rd, 6th and 14th day after the last dose.

In a study of the equilibria between accumulation and elimination of I and II, mice were fed I and/or II for 2 (2 mice), 4 (7 mice), 7 (2 mice), 16 (4 mice), 28 (4 mice), and 60 (18 mice) days. The daily dose of I ranged from 25 to 2500 µg, that of II from 0.78 to 2300 µg (2-5 mice at each dose). This corresponds to average levels in the total feed from 6.7 to 670 mg I/kg and from 0.21 to 620 mg II/kg. The mice then received uncontaminated feed for 5 days and were killed on the 6th day.

In order to estimate the elimination rates of I and II, 15 mice were fed contaminated feed during 4 days with a daily dose of 600 µg I and 320 µg II. After 5, 8, 11 or 14 days on uncontaminated feed, the animals were killed, and the levels of I and II in the abdominal fat were determined. In addition, 11 mice were fed contaminated feed as above, but instead of killing the mice 1/4 of the abdominal fat was removed from each mouse 2, 3 or 4 times for analysis. The operations took place on the 6th, 9th, 12th and/or 15th day after the last dose.

Compounds III and IV were given to 2 mice for 58 days and to 6 mice for 60 days. After 5 days on uncontaminated feed the animals were killed. The daily doses are shown together with the results, Table 3.

The concentrations of I and II in the fat were determined by gas chromatography. The extraction and clean-up were performed as described for organochlorine pesticides (Norén and Westöö 1968). A Pye Unicam GCV with a ^{63}Ni electron capture detector and a glass column (2 mm x 2 m) packed with 3% Dexil 300 or 5% OV 101 on Chromosorb W DMCS, 100/120 mesh, was used for the determinations.

The analysis of the mixture of III and IV added to the feed was performed according to Wells (1979).

RESULTS AND DISCUSSION

The times required from the consumption of I and II to their incorporation into the abdominal fat were studied in succes-

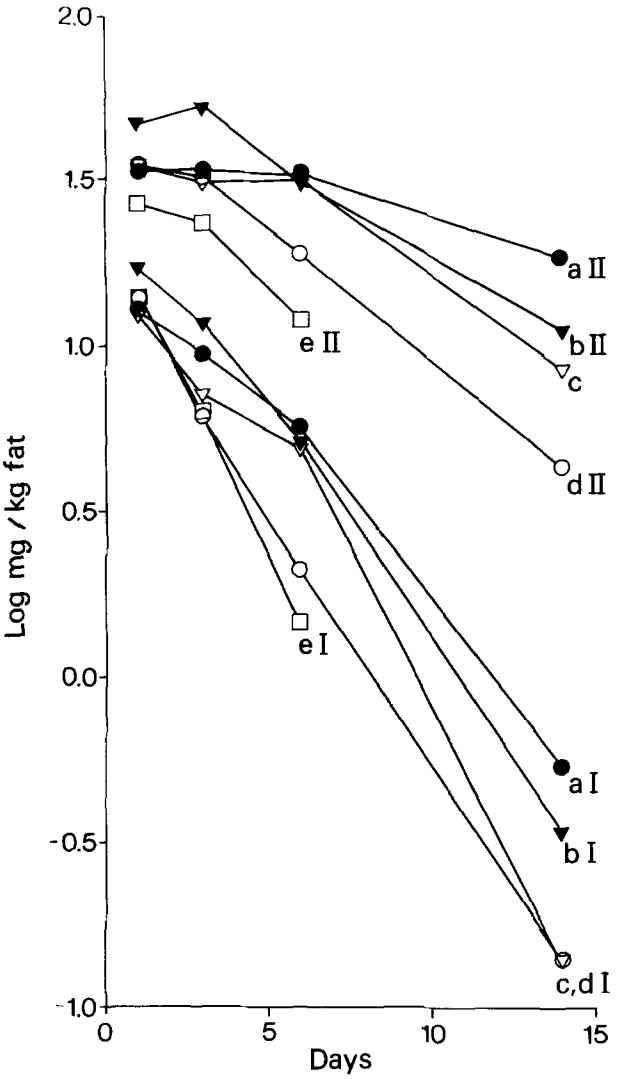

Figure 2. The logarithms of the concentrations (mg/kg) of I and II in fat in mice (a-e) as functions of the time elapsed since the last dose.

sive samples of fat taken from each of 5 mice treated as described above. By studying the changes in the levels of I and II in the fat of each individual mouse, errors due to differences in absorption efficiency between the mice were eliminated. Other advantages of this method are that smaller amounts of compounds I and II and fewer animals are required. However, the operations - though they did not seem to affect the condition of the mice - might influence the elimination rates. Figure 2 shows the logarithms of the levels as functions of time after the last dose. The curves indicate that the incorporation of at least compound II into the fat may have continued for several days after administration had finished. In order to minimize errors caused by the delay, in subsequent experiments mice were fed uncontaminated feed for 5 days before sampling took place.

The equilibria between accumulation and elimination of I and II were investigated in animals given different doses of I and II during 2-60 days. In Figure 3 the average concentrations of accumulated I and II in abdominal fat divided by the daily doses are given as functions of the duration of administration.

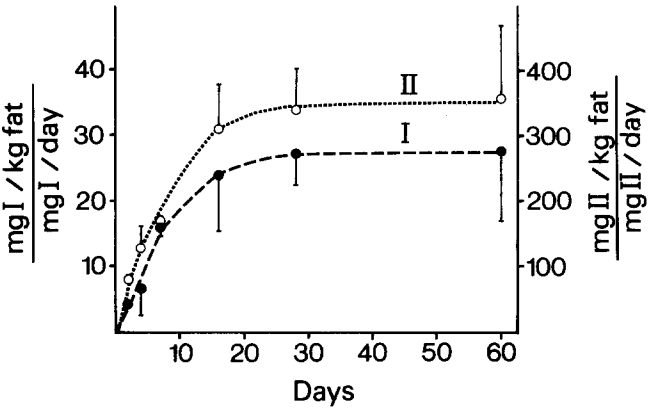

Figure 3. Average concentrations of accumulated I and II in the abdominal fat of mice (5 days after the last dose) per mg of the daily dose as functions of the duration of administration. Each point represents 2, 4, 7 or 18 mice. Bars indicate standard deviations.

The accumulation in fat is assumed to be determined by the equation

$$\frac{d(c_{fat})}{dt} = k_1 c_{feed} - k_2 c_{fat} \qquad (1)$$

where c_{fat} = concentration of the substance in abdominal fat

c_{feed} = concentration of the substance in feed

t = time

k_1 = constant for accumulation

k_2 = constant for elimination

At equilibrium $\frac{d(c_{fat})}{dt} = 0$ and thus $\frac{(c_{fat})_{equil.}}{c_{feed}} = \frac{k_1}{k_2}$ (2)

According to equation 1, constant fractions of the contaminants in the fat are eliminated per unit time after the feeding of these compounds was completed ($c_{feed} = 0$). Thus the characteristics of the curves in Figure 3 should not have changed when all the animals were fed uncontaminated feed for equal periods (here 5 days) after feeding the contaminants. Therefore, as the average c_{feed} is proportional to the daily dose, Figure 3 proves that fairly good equilibria were obtained during the experiments. The curves also show that equilibria were reached after about 30 days and that on the 6th day after the last dose the concentration of II in the abdominal fat was more than ten times that of I.

Taking logarithms of both sides of equation (2) we obtain

$$\log (c_{fat})_{equil.} = \log \frac{k_1}{k_2} + \log c_{feed} \quad (3)$$

Thus $\log (c_{fat})_{equil.}$ should be a linear function of $\log (c_{feed})$ or of log (daily dose), and the slope of the straight line should be 1. If uncontaminated feed is given after equilibrium is established, only parallel shifts of the straight lines should take place. From the individual c_{fat} values of the mice after 28 or 60 days on contaminated feed and 5 days on uncontaminated feed and from the corresponding c_{feed}-values, the equations of the regression lines were calculated by the method of least squares. They were found to be

$\log (c_{fat})_{equil.} = 0.99 \log c_{feed} - 1.03$ for compound I and

$\log (c_{fat})_{equil.} = 1.01 \log c_{feed} + 0.096$ for compound II.

The corresponding correlation coefficients were 0.98 for I and 0.99 for II. This proves that accumulation and elimination of I and II are first order processes as assumed in equation 1.

The elimination rates of I and II from fat were studied in two groups of mice. In the first group the animals were killed at different times after the last dose. In the second group the animals were operated and part of the abdominal fat was removed 2, 3 or 4 times for analysis. The levels of I and II on different days are shown in Table 1.

Table 1. Average levels of I and II in the abdominal fat of mice on different days after the last dose of I and II (ranges in parentheses).

Animals	Day	Number of mice	Compound I, mg/kg fat	Compound II, mg/kg fat
Mice sampled once	6	5	3.0 (1.7-4.7)	33.0 (26.2-39.2)
	9	3	1.8 (0.99-3.1)	23.0 (21.1-24.7)
	12	4	0.35 (0.12-0.52)	8.2 (1.6-12.4)
	15	3	0.10 (0.01-0.18)	4.8 (1.2-6.9)
All the mice	6	10	3.4 (1.5-5.7)	29.3 (12.1-39.2)
	9	6	1.13 (0.19-3.1)	18.2 (10.6-24.7)
	12	8	0.24 (0.10-0.52)	6.1 (1.6-12.4)
	15	11	0.10 (0.01-0.19)	3.85 (0.98-7.1)

When $c_{feed} = 0$, the elimination constant k_2 is obtained from equation (1) by integration.

$$\log (c_{fat}) = \log (c_{fat})_a - \frac{k_2(t-t_a)}{2.303} \qquad (4)$$

where a is a sample taken at the time t_a.

The elimination constants calculated for mice sampled once and for operated mice did not differ much from each other (Table 2). The k_2 values found corresponded to half-lives of 1.6-1.8 days for I and 2.7-2.9 days for II.

Table 2. Elimination constants (k_2) and correlation coefficients (r) for compound I and II in abdominal fat of mice.

Animals	Compound I		Compound II	
	k_2	r	k_2	r
Operated mice	0.38	-0.89	0.24	-0.78
Mice sampled once	0.43	-0.89	0.26	-0.83
All the mice	0.40	-0.89	0.25	-0.80

When the main active ingredients of Eulan WA Neu (compounds III and IV) were given to mice, the compounds I and II were found in the abdominal fat, Table 3.

Table 3. Levels of compounds I and II in the abdominal fat of mice fed different doses of III and IV for 58 or 60 days and uncontaminated feed for 5 days.

mg compound in feed/day		mg compound/kg abdominal fat	
III	IV	I	II
0.079	0.49	0.10	16.9
0.108	0.67	0.17	18.2
0.117	0.055	0.97	3.7
0.117	0.055	0.94	2.7
0.240	0.112	0.79	5.3
0.240	0.112	1.12	4.5
0.49	0.229	2.15	9.6
0.67	0.312	2.93	11.0

The daily doses of I and II required to give the same concentrations in fat as in Table 3 were calculated either from the curves in Figure 3 or from the equations for the regression lines. These doses corresponded to hydrolysis of 7-44% of III and 10-25% of IV. However, the significance of these values is uncertain since the condition of mice fed III and IV was obviously affected. The levels of III and IV were not investigated.

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REFERENCES

Norèn K, Westöö G (1968) Determination of some chlorinated pesticides in vegetable oils, margarine, butter, milk, eggs, meat, and fish by gas chromatography and thin-layer chromatography. Acta Chem Scand 22:2289-2293.

Wells DE (1979) The isolation and identification of polychloro-2-(chloromethylsulphonamide) diphenyl ether isomers and their metabolites from Eulan WA New and fish tissue by gas chromatography-mass spectrometry. Anal Chim Acta 104:253-266.

Westöö G, Norèn (1977) Polychlorinated 2-aminodiphenyl ethers in fish. Ambio 6:232-234.

Westöö G, Norèn K, Egestad B, Palmèr L, Blomkvist G (1983) Structures and ring closures of compounds from the mothproofing agent Eulan WA Neu. Anal Chim Acta 155:293-298.

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