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Translation of two Benthe articles.

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Rough Draft

Translation

ABSORPTION AND DISTRIBUTION OF POLYCHLORINATED BIPHENYLS (PCB) AFTER INHALATORY APPLICATION*

By H. F. Benthe, J. Knop, and A. Schmoldt

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ABSTRACT

In male rats, after a single exposure to aerosol of a PCB-mixture of low chlorinated biphenyls (Pydraul A 200, Monsanto), absorption and distribution were studied by measurements of PCB concentration in different organs. By this method of inhalatory application a very good absorption is shown. Depending on duration of PCB exposure we observed a rapid increase of PCB concentration in liver; the concentration after 15 min. was already more than 50% of maximum concentration attained after nearly 2 hours (70 µg/g tissue). Concentration in fat after 30 min. of exposure is 14 µg/g tissue (about 27% of liver concentration) whereas in brain tissue we found only 9 µg PCB/g tissue (= 17% of liver concentration) at this time.

Dependent on time passed after the end of aerosal application we measured a rapid increase of PCB concentration in liver during 24 hours, accompanied by an increase in levels in brain and fat.

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In the course of 2 days brain and liver concentration fall to a minimum value, whereas in fat a maximum is reached (200 ug/g tissue), which remains constant afterward.

There was no toxic fatty degeneration of liver under these experimental conditions.

Explanation of PCB accumulation in liver and distribution from organ to fat is discussed.

Polychlorinated biphenyls (PCB) have found a wide commercial application as plasticizers in the production of plastics and lacquers, as a cooling agent and insulation material in electrical engineering, and as nonflammable, anhydrous hydraulic fluids in mechanical conveying, for example, in mining. Depending on the physical requirements, mixtures with a chlorine content of 30-65% (di-bis-heptachlorobiphenyl) have been used.

A few investigations of the toxicological properties of these substances have been available so far. Drinker et al. (1937) found that addition of the PCB vapors to the air in chronical tests with rats caused progressive liver damage, which Treon et al. (1956) were able to confirm after oral and Miller (1944) after percutaneous absorption in guinea pigs, rats, and rabbits. Chlorine-acne-like contact dermatitis was observed in people (Meigs et al., 1954). Further toxicological investigations, in view of the wide spread application, appear to be particularly required because, since 1966 in the residue analyses, besides pesticides, PCB are also found. They are found in the coastal water of North America (Risebrough et al., 1968; Duke et al., 1970) and of Northern Europe (Holmes et al., 1967; Holden and Marsden, 1967; Koeman et al., 1969) in water, ocean floor, shells, algae, fish, sea mammals, and fish-fed birds and their eggs.

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PCB's have also been detected in adipose tissue and in the mother's milk of humans; the concentrations are in the same order of magnitude as that of DDT (Biros et al., 1970; Acker and Schulte, 1970).

In the commercial application of PCB's as nonflammable hydraulic liquids, the inhalation of the liquids presents the most frequent exposure form for man. During technical disorders (blow-out of superheated couplings, leakages in pressure systems) very stable aerosols of these compounds are formed. In this manner extremely high concentrations in closed spaces may occur for relatively long periods of time.

Since most toxicological investigations were carried out to date after oral, intraperitoneal, and cutaneous exposures to PCB, it appeared necessary to clarify the toxicological properties after an aerosol application in the animal tests. For these tests we used the PCB mixture Pydraul A 200[R1] (in the following referred to as A 200) which is very frequently used as a hydraulic fluid in mechanical conveying. Its chlorine content amounts to 42%.

Materials and Methods

The tests were carried out on male Wistar rats (220-300 g), which 15-20 hours before the tests were deprived of food (Altromin). The aerosol was produced in an aerosol generator (Drager; particle size 0.5-3μ at 3-5 atm) at 150°C. After passing through an empty wash bottle for cooling and separation of larger particles, the aerosol was conveyed through a short hose (20 mm diameter) into a closed rectangular plexiglass chamber (43 x 81 x 37 cm). The inlet and outlet connections (20 mm diameter) were located near the floor in the opposite narrow sides of the chamber. As an animal container for a maximum 6 rats we used a wide-meshed lattice bar cage (plexiglass; 21 x 33 x 22 cm) which was placed in the spacial center of the aerosol chamber described above.

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1 We acknowledge the generous donation of the substance by Monsanto Co.

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For the entire duration of the test the generator supplied a very stable uniform aerosol, and the air throughput amounted to 1 m^3/hr. To determine the aerosol concentration, a preheated cylinder (1.5 l) was placed in the chamber, which after about 30 min. was closed and immediately was cooled in an ice-salt mixture. The precipitated PCB was dissolved in heptane, and the PCB concentration was determined photometrically in an aliquot part. The PCB determination carried out at different intervals of the experiment gave a uniform value of 30.4 ± 3.4 g/m^3.

After the exposure to the aerosol, the animals were killed either immediately or after a given time by a blow in the neck and decapitation. To that moment the animals obtained food and water in the desired quantities.

The extraction of the tissue lipides was carried out either immediately after removing the organs or after storing the organs at -25°C. The liver, before its removal, was rinsed with 0.9% NaCl for a complete blood removal from the abdominal aorta. For the analysis of the adipose tissue only the retroperitoneal adipose tissue was used. Since the lipides are not further separated, the following data for fat and lipide are always based on the total chloroform extract.

A 1.0 g tissue sample was pulverized with anhydrous Na_2SO_4 to a dry powder and was extracted twice with 50 ml $CHCl_3$. The filtered $CHCl_3$ extracts were combined and were concentrated to dryness in a rotary evaporator. The fat determination was carried out gravimetrically after drying over $CaCl_2$ until a constant weight was obtained. About 50 mg of the lipide was weighed exactly and was boiled with 10% ethanolic potassium hydroxide for 2 hours under reflux and then was evaporated to dryness. The hydrolyzed fat was taken in 3 ml water, and the aqueous phase was extracted with 6 ml heptane. Each time 5 ml heptane was removed, collected, and distilled to dryness in a rotary evaporator. The residue was dissolved in 1.0 ml heptane.

Since gas-chromatographic measurement methods were not available, the heptane solution was fractionated by thin layer chromatography (silica gel with fluorescein indicator Riedel de Haen; eluent: twice with petroleum spirit, b.p. 40-60°C), the spots corresponding to the reference substance were extracted with a total of 5 ml heptane and were measured at 248 nm in Spectrophotometer PMQ 2 (Zeiss). The UV spectrum of the PCB mixture used and those of both spots isolated from the chromatogram show an absorption band between 245 and 250 nm, which is suitable for a quantitative determination. The sum of the individually isolated chromatogram spots gave the same value as that of the same quantity of the known chromatographed starting substance. The sensitivity of the method was entirely sufficient for the investigations; the lower detection limit is 0.3 µg/ml. Moreover, the tin layer chromatography has an advantage because further expensive "cleanup" methods are not required anymore. The yield of the A 200 quantity added to the tissue homogenates (15-75 ug/g tissue) amounted to: 72.6 ± 6.6% for liver, 75.1 ± 3.1% for brain, and 69.1 ± 8.8% for adipose tissue. For the calculations, a uniform value of 72% was used.

To determine the glycogen contents of the liver, 400 mg tissue was boiled with 3 ml 4 N KOH for 20 min. and then was centrifuged. The sediment was suspended in 3 ml 96% ethanol and was reprecipitated at the boiling temperature. The glycogen was hydrolyzed with 2 ml 4 N HCl at 100°C during 60 minutes, the hydrolysis product was neutralized with KOH and K_2CO_3 and was filled to 100 ml. The determination of glucose thus obtained from glycogen was carried out enzymically with hexokinase, glucose-6-phosphatedehydrogenase,,and NADP, by measuring the resulting NADPH at 366 nm.

All the chemicals were of the analytical grade, the organic solvents were spectrally pure (Uvasol Merck), the glucose determination was carried out by the glucose test combination (Boehringer Mannheim).

The statistical evaluation of the tests on 4-6 animals was carried out according to the uncombined t-test. An error probability of 5% is assumed.

RESULTS

Since PCB's are considered as toxic to liver, the question of the absorption of Pydraul A 200 in the liver was the central point of the investigation.

As Figure 1 shows, an exposure time of 15 min. to aerosol was sufficient for achieving more than 50% of the maximum deposition in the liver. After that the absorption curve has a considerably flatter course and after 2 hours approaches the value of 69.7 µg/g liver wet weight, the maximum concentration. Knowing this rapid absorption by the liver, the exposure time in the following investigations was limited to 30 minutes.

In the investigation of the questionhow long the applied PCB mixture remains stored unchanged in the liver, the test animals were killed not immediately after the exposure to the aerosol, but after 12 and 24 hours, respectively. During that time the rats were fed again. Since feeding after 24 hours fasting (before the exposure to the aerosol) leads to physiological changes of the liver weight and of the fat contents, the data of the A 200 concentration were given in µg/g liver lipide.

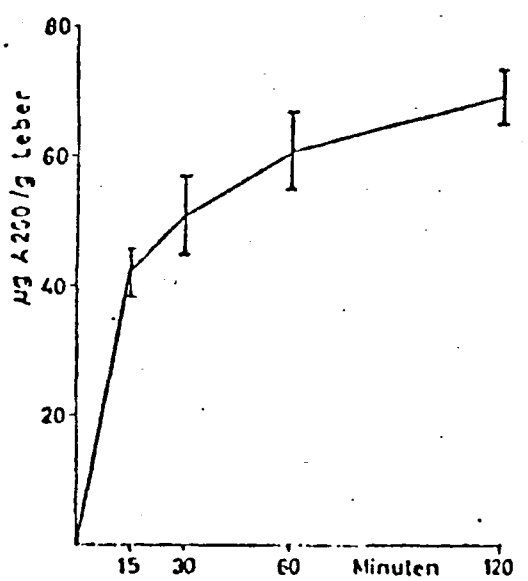

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Figure 1. Absorption of A 200 in the liver as a function of the exposure time. The average value of 6 rats ± sx.

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Figure 2 shows the concentration decrease as a function of time. After 12 hours the contents decreased already to less than a half of the starting quantity. After 2 days the PCB concentration fell to a value of 96 µg/g liver lipide.

These findings left open the question whether this involves a metabolic process, excretion, or a redistribution into the other organs.

Since PCB's are highly lipophilic substances which are found, besides pesticides, in the animal adipose tissue, the retroperitoneal adipose tissue of rat was analyzed under the same experimental conditions as in the liver tests. It was established thereby that immediately after the aerosol exposure with 19.3 µg/g lipide (corresponding to 14 µg/g adipose tissue) only traces of PCB were detected in the adipose tissue. After 12 hours only a slight concentration increase is measurable, and only after 36 hours the A 200 contents reaches a maximum of 352 µg/g fat. After 72 hours this value is not essentially lower (Figure 3). These findings speak against the only elimination by the liver that has not been clear, rather it appears that first a transfer into the adipose tissue takes place.

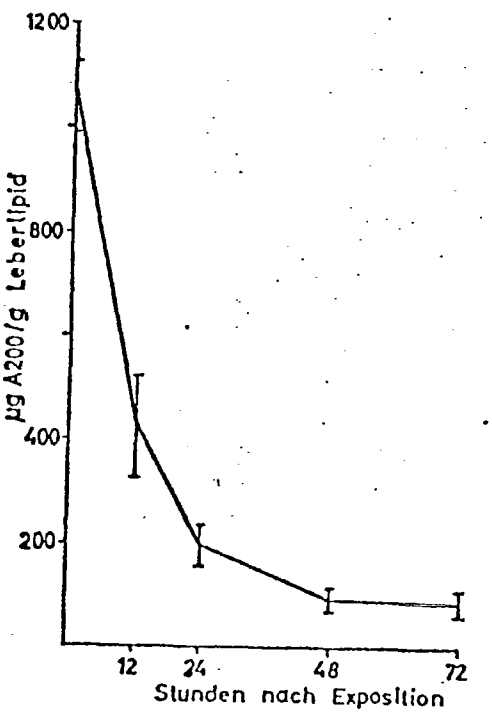

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Figure 2. The A 200 concentration decrease of the liver after 30 minutes aerosol exposure as a function of time. The average values for 6 rats ± sx.

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As in the case of DDT, it is here also of a particular toxicological interest to find out whether PCB's can be detected in the brain. Again the same experimental conditions were chosen. In contrast to the findings in the adipose tissue, immediately after the exposure to A 200, the latter could be detected in the brain. The measurements as a function of time after the exposure (Figure 4) gave a concentration increase reaching a value of 226 µg/g brain lipide after 24 hours. After that there occurred a decrease down to the concentration equilibrium with the liver lipide.

To verify whether A 200 in the form of the inhaled aerosol leads to a toxic fatty degeneration of liver, the liver fat contents was determined as a function of the exposure time and was compared with the control animals. It was necessary thereby to observe that the control animals also remained fasting during 24 hours, since the fat contents increased during fasting (Ekmann, 1949).

It was verified by means of the liver glycogen contents that here a dissimilating metabolism condition was involved, since after 24 hours of fasting the glycogen contents decreased to zero (in normally fed control animals 3.5 ± 0.9 g %). As another conditionally valid parameter of a liver degeneration, the relative liver weight was measured. Table 1 shows that even an aerosol exposure of 2 hours does not lead to any significant change either in the liver fat contents or in the relative weight of the liver, as compared with control animals.

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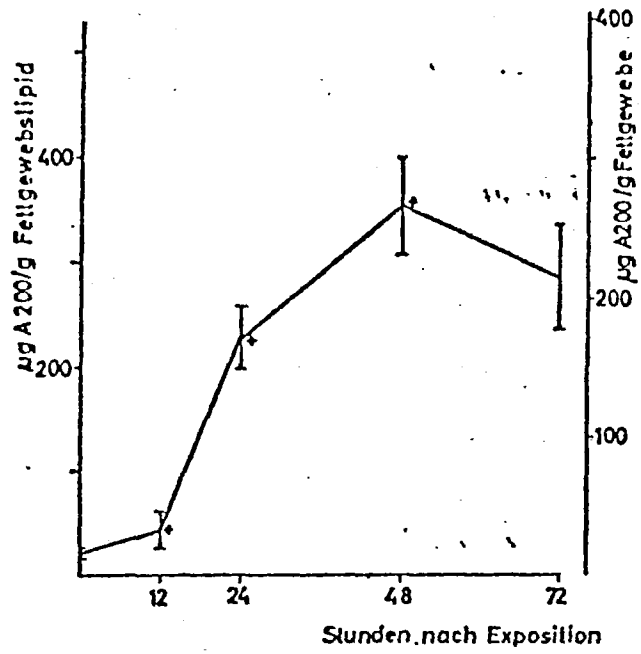

Figure 3. The concentration course of A 200 in the adipose tissue after 30 min. aerosol exposure as a function of time. The average values of 6 rats ± sx. + = a significant difference of the adjoining values.

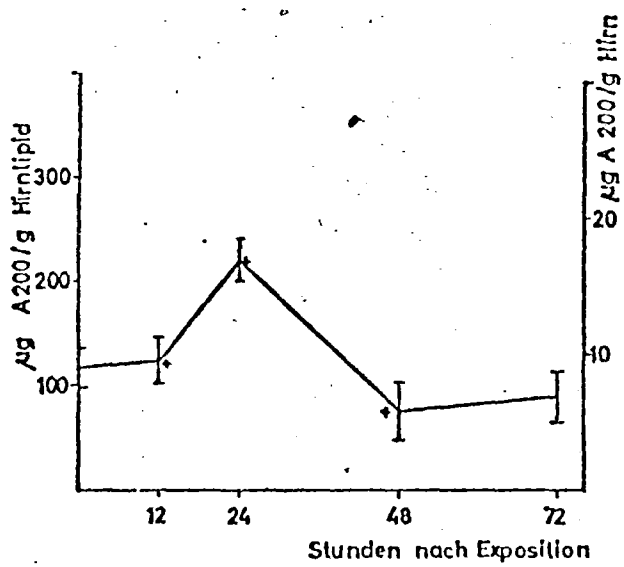

Figure 4. The A 200 concentration in the brain as a function of time after 30 min. aerosol exposure. The average values of 5 rats ± sx. + = a significant difference of adjoining values.

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Table 1. The behavior of the liver adipose contents (g %) and of the relative liver weight (g % body weight) as a function of the exposure duration to A 200 aerosol. The average values of 6 rats $\pm$ s.

	Control Normally Fed	Control 24 Hr. Fasting	Duration of the Aerosol Exposure 15 Min.	30 Min.	60 Min.	120 Min.
Liver Fat (g %)	3.6 $\pm$ 0.4	4.9 $\pm$ 0.8	4.7 $\pm$ 0.6	4.8 $\pm$ 0.5	4.3 $\pm$ 0.5	5.1 $\pm$ 0.1
Relative Liver Wt. (g %)	3.8 $\pm$ 0.3	2.8 $\pm$ 0.1	3.1 $\pm$ 0.5	2.9 $\pm$ 0.2	3.0 $\pm$ 0.1	3.0 $\pm$ 0.2

Table 2. The behavior of the liver adipose contents (g%) and of the relative liver weight (g % body weight) after 30 min. aerosol exposure as a function of time. The average values of 6 rats $\pm$ s.

	Control 24 Hrs. Fasting, 24 Hrs. Feeding	Control 24 Hrs. Fasting	Time After 30 Min. Exposure to Aerosol 0 Hr.	12 Hrs.	24 Hrs.	48 Hrs.	78 Hr
Liver Fat (g %)	3.0 $\pm$ 0..2	4.9 $\pm$ 0.8	4.8 $\pm$ 0.5	3.4 $\pm$ 0.4	2.9 $\pm$ 0.3	2.8 $\pm$ 0.4	3.0 $\pm$ (
Relative Liver Wt. (g %)	4.5 $\pm$ 0.4	2.8 $\pm$ 0.1	2.9 $\pm$ 0.2	5.0 $\pm$ 0.3	4.2 $\pm$ 0.5	4.0 $\pm$ 0.4	4.3 $\pm$ (

To verify whether the liver changes occur perhaps after a certain delay, the parameters were followed up to 72 hours after an exposure of 30 minutes. The values given in Table 2 do not differ from the control data. The decrease of the lipide values to the subnormal values after the renewed feeding can be explained as a build-up process.

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DISCUSSION

During suddenly occurring leakages in the hydraulic pressure system (approximately 120 atm.) and in heat exchangers maintained under pressure there are formed very stable aerosols to which the operating personnel may be exposed for a longer time. To imitate such situations in model experiments, rats were exposed only once for a relatively short time to the PCB aerosol. In addition, these tests had the advantage of allowing us to study the kinetics of the PCB distribution. The rapid accumulation in the liver and the measurement of the maximum concentration immediately after the exposure indicate that PCB enters immediately into the blood circulation and is not deposited first in the alveoli (see Figures 1 and 2).

Because of the lipophilic properties and the structural relation to DDT, above all the distribution was investigated in those organs which are also of main toxicological interest for DDT and similar pesticides. These organs include brain, liver, and adipose tissue (Kagan, 1969). In the previous investigations there was reported particularly about the liver toxicity of PCB's (Drinker et al., 1937; Treon et al., 1956; Miller, 1944). The investigations which were primarily pathohistological left out, however, the quantitative PCB analyses of the organs.

While during the enteric resorption a high PCB concentration in the liver is to be expected, it was surprising to also find the highest concentration in the liver during the aerosol exposure. In contrast, in spite of the many times higher blood circulation, we found in the brain only 11% of the liver concentration (based on ppm lipides) and 17% (based on ppm wet tissue). Immediately after exposure, only traces of PCB are detectable in the adipose tissue. During the time following the exposure, the distribution pattern changed. Within the first 24 hours the PCB content in the liver decreases exponentially, while at the same time the concentration in the brain and in the adipose tissue increases to the same value (based on the ppm lipide). In the brain, however, the contents decreases after 48 hours below the starting value and reaches, like the liver, a level of 80 ppm lipide, which

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in both organs does not change significantly anymore, even after 72 hours. In the adipose tissue the maximum is reached only on the second day after the exposure. The value of 352 ppm does not significantly decrease on the third day findings can be explained only as a redistribution of PCB from the liver into the adipose tissue.

It is not clear why the applied PCB mixture initially accumulates predominantly in the liver. The higher blood circulation through the liver alone cannot explain the accumulation. The brain which has even better blood supply reaches, during the 30 minutes lasting exposure, only 17% of the concentration that is measured in the liver tissue. The brain concentration increases, however, in the phase of the redistribution from the liver into the adipose tissue, although the plasma concentration is then lower than at the time of exposure. It is possible that for the explanation of the PCB enrichment in the liver one may consider the condition that the rats were fasting for 20 hours prior to the test. Perhaps this period of time is sufficient for adjusting the metabolism to the conditions of fasting. For the fat metabolism this means an increase of lipolysis with simultaneously inhibited absorption of triglycerides in the adipose tissue.

The nonesterified fatty acids set free combine with plasma albumen and are absorbed by the liver where they serve as the energy source and, due to an inhibited lipoprotein synthesis, are deposited in increased quantity as triglycerides (Tarnowski and Seitz, 1970). Parallel to the increased triglyceride concentration of the liver, an increased PCB absorption may occur. Preliminary results of the investigations with fed rats, under otherwise the same experimental conditions, confirm this hypothesis so far as the PCB concentration of the liver at the beginning was actually found somewhat lower, nevertheless it is still higher than in the other organs.

Nothing has yet been published on the PCB redistribution from the liver into the adipose tissue. Grant et al. (1971) reported about the distribution after oral

application and found, in the case of this longer lasting absorption in the adipose tissue immediately from the beginning of the experiment, higher values than those of the liver.

It is also not known to what extent, besides the redistribution, the elimination and metabolic processes take place. According to the investigations of the enteric resorption in the presence of the catheters introduced into the bile duct and into the bladder, within the first 9 hours no excretion of unchanged PCB could be detected (Benthe and Schmoldt, in preparation). It is known about the elimination of PCB's after a prolonged period of time that the compounds with a lower chlorine content are more rapidly eliminated than are the higher chlorinated biphenyls (Grant et al., 1971).

The liver toxicity cited above is manifested in a central atrophy and an adipose degeneration as well as in a porphyry (deVos and Koeman, 1970). Under the experimental conditions of a single, short-lasting aerosol exposure there was not found, however, either a decrease of the lipide contents or an increase in the relative liver weight. Likewise, no pathological changes were detectable histologically by light microscopy. However, other investigations (Nissen, 1971) indicate that during longer exposure times the fat contents of the liver increases and the liver functions are pathologically changed.

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