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Disturbance of Motor Performance and Thermoregulation in Mice Given Two Commercial Chlorinated Paraffins

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Chlorinated paraffins (CP) are chlorinated derivatives of straight aliphatic hydrocarbon chains, ranging between 10 and 30 carbon atoms, generally chlorinated from 40 up to 70 % by weight (Hardie 1964). Commercial CPs contain added stabilizers (Hardie 1964; Howard et al. 1975) and they may also hold such impurities as chlorinated isoparaffins and chlorinated aromatic hydrocarbons (Zitko and Arsenault 1974; Zitko 1980).

The major part is used in plastic products such as fire retardants and plasticizers, and as additives in lubricant and cutting oils. Thus they have, in many respects, become a substitute for polychlorinated biphenyls (PCB). The world consumption of CPs in 1977 was estimated to be about 230 kton (Campbell and McConnell 1980).

The CPs have been shown to be present in the environment (Campbell and McConnell 1980), although in very small quantities, and must be regarded as potential environmental hazards.

In mammals, the CPs show a low acute oral toxicity with a LD_{50}--value above 4 g/kg body weight in rats (Birtley et al. 1980; Howard et al. 1975). However, there are some known data concerning toxic effects and accumulation of CPs in fish (Svanberg et al. 1978; Bengtsson et al. 1979; Madley and Birtley 1980). These data indicate a motor disturbance and an accumulation of organic chlorinated compounds. The effects are partley related to carbon chain length and chlorination degree.

The aim of the present study was to measure motor capacity in mice, after an intravenous injection of CPs with different degree of chlorination. In addition, the body temperature was measured, because it is known that some substances, (CNS-depressants and major tranquilizers), which decrease the motor capacity also decrease the rectal temperature in mice (Campbell and Richter 1967).

MATERIALS AND METHODS

Two types of CPs were used, Cereclor 50LV® (Imperial Chemical

Industries Limited, England) (10- to 13-carbon n-paraffins, chlorinated to 49 % on average, m.w. av. 330) and Cereclor 70L® (Imperial Chemical Industries Limited, England) (10- to 13-carbon n-paraffins, chlorinated to 70 % on average, m.w. av. 530). The lipids and emulgators, which are constituents of Intralipid® (Kabi-Vitrum, Sweden) (total concentration 10 %), were used by Roland Jeppsson (Kabi-Vitrum, Sweden) to prepare two stock emulsions containing 37.5 and 3.75 mg CP/ml, respectively. The former was diluted to 29.0, 20.6, 12.2 mg CP/ml with Intralipid (100 mg/ml).

Sixty adult male mice (21-30 g), NMRI, (Anticimex, Sweden) were divided into 12 equal groups. The animals were supplied with standardized pellet food (Ewos, Sweden) and tap water ad libitum before and throughout the experiment. One day prior to the injections the mice were placed in separate cages. The mice were given a CP (Cereclor 50LV or Cereclor 70L) by an intravenous injection in the tail vein. The dose levels were 300, 232.5, 165, 97.5, 30 mg/kg body weight. Two control groups were used to compensate for different amounts of lipophilic substances in the emulsions. Thus, the control animals received in the same manner 8 ml emulsion/kg body weight of 100 and 200 mg Intralipid/ml, respectively.

An accelerating rotarod (cf Jones and Roberts 1968) was used to measure the motor capacity in mice. The method is a modified form of a constant speed rotarod (Dunham and Miya 1957). The rotating rod consists of a horizontally placed rubber coated bar (3.18 cm in diameter, and with 20 cm free space below the bar). The mouse was placed between two opaque vertical adjustable discs, about 5 cm apart, which prevented it from changing position and running direction. The acceleration was almost linear (from 0 r.p.m. to 50 r.p.m. over a period of 70 seconds). Each animal was given a single trial to remain on the accelerating rotarod and the r.p.m.-value was read as soon as the head of the mouse was below the bar. The r.p.m.-value of each untreated mouse was registered on the day before the injections, and this value was compared with the r.p.m.-value after injection. In preliminary experiments with twentyfive untreated mice, all animals, from one day to the other, improved their ability to remain on the accelerating rotarod when they were allowed only a single trial a day. Thus, impaired or unchanged r.p.m.--values obtained after injection indicate a decreased motor capacity. Mice with improved ability were marked with positive signs and those with unchanged or reduced ability with negative signs. The mice were tested at 15 minutes (Cereclor 50LV) and 40 minutes (Cereclor 70L) after the respective injection. In preliminary experiments, the time chosen was found to be the most sensitive for measuring rotarod performance. The rectal temperature was registered with an electric thermometer, just before the injection, and 30 and 60 minutes later.

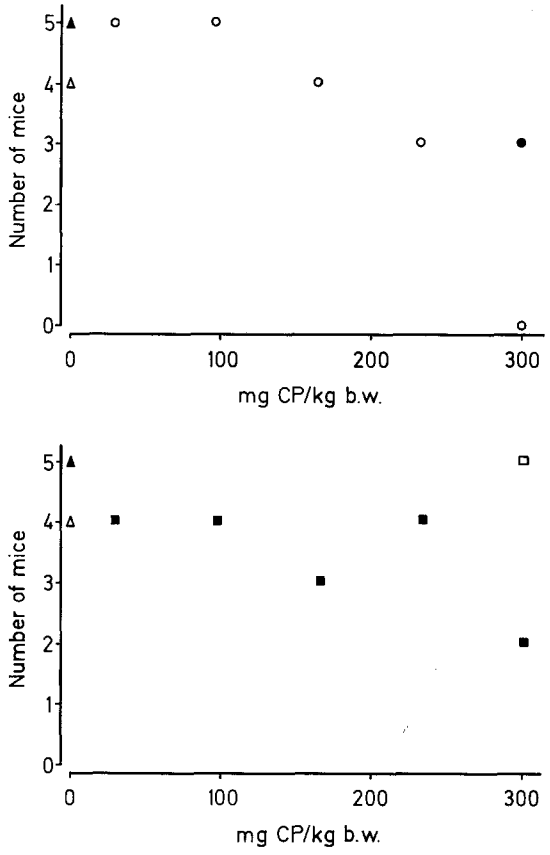
Statistical evaluation of the frequencies of positive and nega-

tive signs were performed by Fishers exact probability test. Comparisons of the temperature results were performed by Mann--Whitneys U-test.

RESULTS AND DISCUSSION

The vehicle-injected control mice showed an improved motor capacity and no change in rectal temperature.

In mice given Cereclor 50LV or Cereclor 70L there is a statistically significant decrease ($p<0.025$) of the motor capacity in mice receiving the highest dose (300 mg/kg b.w.) of Cereclor 50LV and there is a tendency towards decreasing motor capacity with increasing doses (fig. 1). One possible expla-



A. Cereclor 50LV, rotarod test 15 (○) and 40 (●) min. after injection, respectively.

B. Cereclor 70L, rotarod test 40 (■) and 15 (□) min. after injection, respectively.

Fig. 1. Number of mice with improved motor capacity after an intravenous injection of a CP. Each point represents the number of mice in a group of five mice, whose ability to remain on the accelerating rotarod was improved. The controls were tested 15 (△) min. (Intralipid 100 mg/ml) and 40 (▲) min. (Intralipid 200 mg/ml) after injection, respectively.

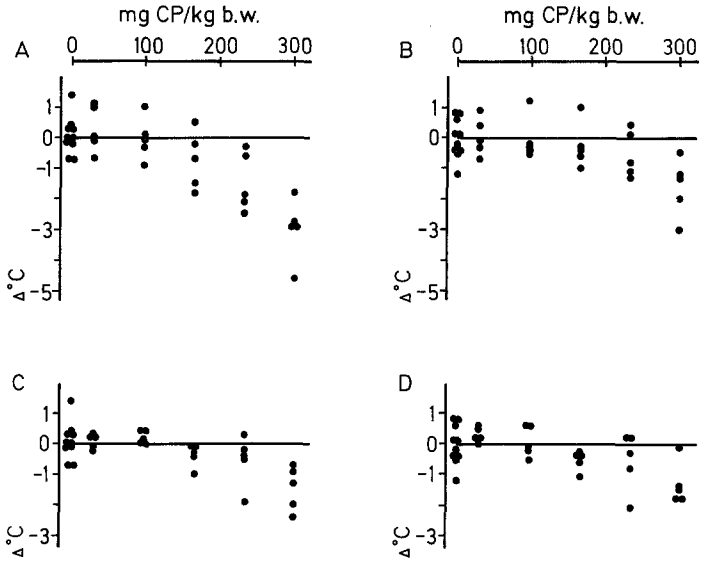


Fig. 2. Change in rectal temperature in mice after an intravenous injection of a CP.
A. Temp. diff. ($\Delta^{o}C$), 30 min. after an inj. of Cereclor 50LV
B. " " " , 60 min. " " " " "
C. Temp. diff. ($\Delta^{o}C$), 30 min. after an inj. of Cereclor 70L
D. " " " , 60 min. " " " " "

nation for the more pronounced effect caused by Cereclor 50LV can be the lower molecule weight given a higher molarity of this substance.

The thermoregulation was affected in mice receiving Cereclor 50LV and Cereclor 70L. A statistically significant decrease in the rectal temperature ($p<0.05$) was observed at the highest dose (300 mg/kg b.w.) for both substances, and there is a tendency towards decreasing temperature with increasing doses (fig. 2).

The effects were manifest only in high CP doses. Darnerud and Brandt (1982) have shown that after an intravenous or oral administration of a terminally ^{14}C-labelled CP of low chlorine content ($C_{16}H_{30}Cl_{3.3}$) a high proportion of ^{14}C was found as $^{14}CO_2$ in the expired air (about 45 %, twelve hours after the administration). It may be that the low toxicity in mammals is due to a rapid metabolization of the compound. However, the chlorination degree may be of importance. In contrast to Birtley et al. (1980), who could not observe any difference in effect between CPs of low and high chlorine content, the rotarod results indicate the opposite. Mice treated with Cereclor 70L (300 mg/kg b.w.) showed no reduced motor capacity 15 minutes after injection, which, however, was the case for mice given Cereclor 50LV. It may be that the uptake into target cells and/or the metabolization of the compounds are of importance.

The higher dose levels of both CPs, an unwarranted cessation of movement, with one forepaw in the air, during normal walking was particularly noticeable in the mice.

From the rotarod test and temperature measurements, it is seen that the effects could only be observed at high doses of CPs. This is in agreement with other toxicity studies concerning CPs in mammals (Howard et al. 1975; Birtley et al. 1980). However, the difference in effect between low and high chlorinated paraffins should be investigated further.

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