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Metabolism of Pentachlorophenol to Tetrachlorohydroquinone by Human Liver Homogenate

U. Juhl,[1] I. Witte,[1] and W. Butte[2]

Fachbereich [1]Biologie and [2]Chemie, Universität Oldenburg, D 2900 Oldenburg, Federal Republic of Germany

Pentachlorophenol (PCP) and its salts are among the most widely used pesticides in the United States (Williams 1982). The greatest amount of PCP is used in the wood preserving industry. Because of the suspected health hazards of PCP the US EPA issued notices of rebuttable presumption against registration and continued registration for the wood preservative use of PCP (Jellinek 1981, EPA 1982). Levels for an acceptable daily intake (ADI) have been established to be 0.003 mg/kg/day. National residue tolerance levels have been set for PCP for a few food stuffs in Germany, the Netherlands and Australia (Ahlborg and Thunberg 1980).

The metabolism of PCP in humans and monkeys as well as in rodents has been investigated. Whereas tetrachlorohydroquinone (TCH) was a major metabolite of PCP in rats and mice (Jakobson and Yllner 1971, Ahlborg et al. 1974), in humans, after ingestion of 0.1 mg PCP/kg body weight, only unchanged PCP and PCP-glucuronide was found. In these experiments the urine was also analysed for TCH, but none was detected (Braun et al. 1979). However, Ahlborg et al. (1974) found TCH in urine of workers occupationally exposed to PCP. Recently, Witte et al. (1985) have shown, that, in contrast to PCP, TCH has genotoxic properties; it binds to DNA, causes strand breaks in DNA, and is more toxic to human fibroblasts than PCP. On the basis of this knowledge we consider it essential to resolve the question, whether or not PCP can be metabolized to TCH in humans. Therefore, we investigated the metabolism of PCP in human liver homogenates and compared it with the metabolic conversion of PCP in rat liver.

MATERIALS AND METHODS

For preparation of S-9 fraction a human liver sample from a 61-year-old woman and rat liver from 8-10 week old male Wister rats were used. Animals were killed

after a 16 hour period of fasting. The S-9 fraction from human and rat liver was prepared according to the method of Ames et al. (1975). Aroclor 1254-induced rat liver S-9 fraction was purchased from Litton Bionetics, Charleston, S.C. Neither of the S-9 fractions contained detectable levels of PCP and TCH.

S-9 mix was prepared immediately before use. The S-9 fraction (final concentration 10 %) was mixed with a solution containing 8 mmol/l $MgCl_2$, 33 mmol/l KCl, 5 mmol/l glucose-6-phosphate, 4 mmol/l NADP, pH 7.1. The protein concentration in the incubation mixtures was 2.5 - 4.0 mg/ml. Protein determinations were made by the method of Lowry.

9 volumes of S-9 mix were incubated at 37 °C up to 24 hours with 1 volume of 0.01 to 1 mmol/l PCP (EGA, 99 %) dissolved in 100 mmol/l sodium phosphate buffer, pH 7.1. Thereafter, protein from S-9 fraction was removed from the incubation mixture by precipitation with an equal volume of 10 % perchloric acid. The amount of TCH produced was determined by gas chromatography after pyrolytic ethylation to tetrachloroquinone-diethylether. This method was already described for PCP and tetrachlorophenols (Butte et al. 1983).

RESULTS AND DISCUSSION

Human liver S-9 fractions metabolize PCP to TCH (Fig. 1). The pharmacokinetics of this reaction corresponds in rate and progression to the metabolism obtained with rat liver homogenates. The maximum of TCH formed in the incubation mix is present after 3 hours, declines to half of this value at 6 hours, and at 24 hours TCH is no longer detectable in the incubation mixtures. The decrease of TCH during the course of incubation may be explained by two processes. On the one hand the oxidation capacity of the S-9 mix gets exhausted, on the other hand TCH is oxidized at neutral conditions via semiquinone radicals.

Although we observed similar activities of human and rat liver homogenates, one should realize that the capacity of human S-9 fractions to metabolize various compounds shows great interindividual differences (Tang and Friedman 1977, Sabadie et al. 1980). To investigate the possibility of an increase in metabolic conversion of PCP to TCH, the experiments described above were repeated with Aroclor 1254-induced rat liver post-mitochondrial fraction. Aroclor 1254 consists of a mixture of polychlorinated biphenyls (PCB's). Fig. 1, inset, shows that the induced S-9 fraction enhances the formation of TCH. At 5 hours there is a 40-fold in-

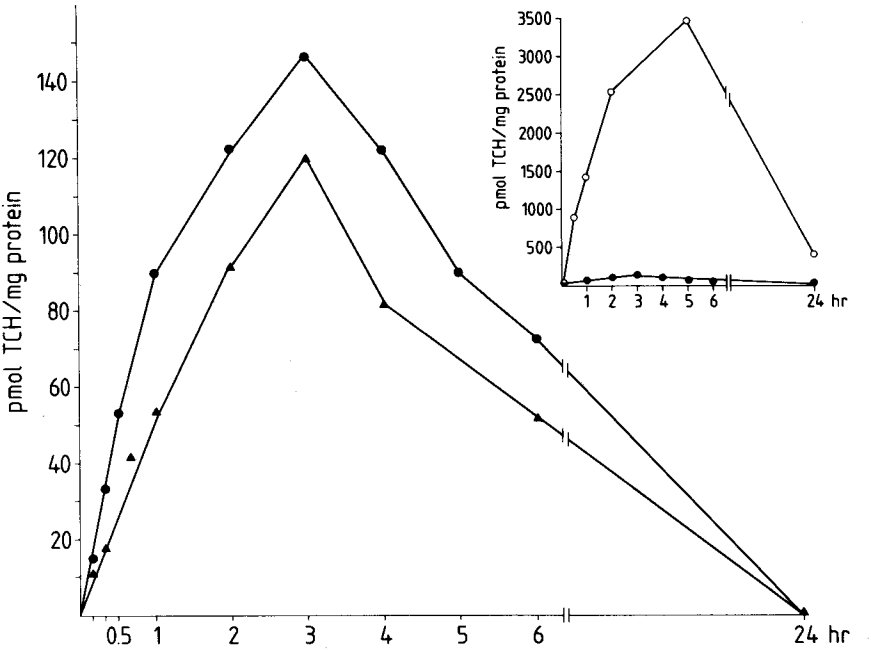

Figure 1. Amount of TCH formed after incubation with S-9 fraction: human (▲) and rat (●) liver. Inset: TCH formed by S-9 fraction of rat liver: induced by Aroclor 1254 (o), not induced (●).

crease of TCH over the noninduced sample (3.5 nmol TCH/mg protein). Ahlborg et al. (1978) had obtained comparable results with phenobarbital-induced rat liver microsomes.

It is known that PCP has an inhibitory effect on certain enzymes of the S-9 fraction. Inhibition of the sulfotransferase (Mulder and Meerman 1978) and of the terminal oxygenation enzyme P 450 have been described (Arrhenius et al. 1977). From the latter findings it may be expected, that PCP impedes its own metabolic conversion to TCH. Fig. 2 shows the rate of PCP metabolism with rat liver S-9 fraction at different PCP concentrations. This rate (µmol TCH/mmol PCP in the assay) is by a factor of 1,000 lower at a concentration of 1 mmol/l PCP than at 0.01 mmol/l. Even though there is inhibition at 0.1 mmol/l PCP, the greatest amount of TCH is actually produced at this concentration.

In comparing the metabolism of PCP in man, monkey and rat, Braun et al. (1979) came to the conclusion that neither the rat nor the monkey are exactly like man,

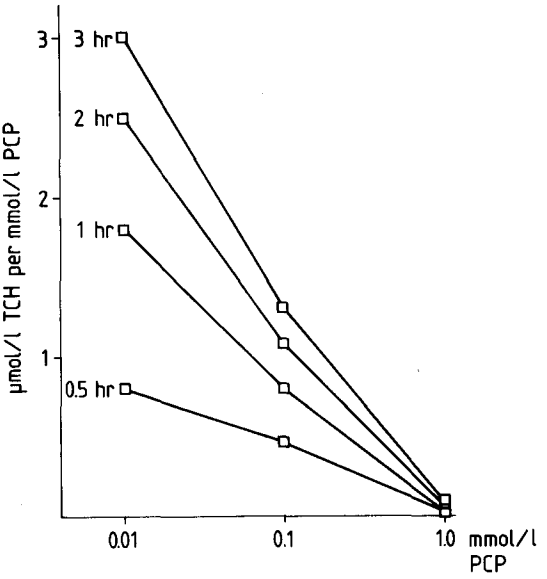

Figure 2. Concentration of TCH with respect to PCP formed at various starting concentrations of PCP.

and that detoxification in man only occurs through glucuronidation of PCP. However, Ahlborg et al. (1974) had actually found TCH in urine of people occupationally exposed to PCP. Our findings demonstrate that PCP can be metabolized to the genotoxic metabolite TCH by human microsomal enzymes. Therefore, the TCH found in human urine by Ahlborg et al. (1974) was most likely the result of PCP exposure.

The quantitative ratio of PCP-glucuronide and TCH is influenced by the activity of oxidizing microsomal enzymes and also by the PCP concentration in the liver inhibiting the formation of TCH. This means that exposure to low levels of PCP and concomitant microsomal enzyme induction would produce highest levels of TCH. Therefore, people who are occupationally exposed to enzyme-inducing PCB's would be the most endangered, because exposure to low levels of PCP from our environment is unavoidable at this point (Ahlborg and Thunberg 1980).

The present ADI-level does not take into consideration that PCP converts into a genotoxic metabolite and that this metabolite is formed in the human liver. With this knowledge a review of the ADI-level becomes necessary.

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