The Development of Tolerance of 3-Chloro-*p*-Toluidine in the Rat (34838)

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The acute toxicity of the avicide 3-chloro-*p*-toluidine (CPT, DRC-1339) has been studied in certain species of mammals and most particularly in the rat (1, 3) and it was found to be only slightly toxic (2).

Once this avicide is in use it is possible that mammals, including man, could be subjected to repeated exposures. The effects of repeated sublethal doses in mice were studied (3) and they are described as follows:

". . . following dosing the animals became ataxic, cyanotic and comatose, this lasting from 30 minutes to 1 hour. It appeared that males were affected more severely and recovered much slower than females. However, with each additional feeding the effects were less pronounced; after 4–5 days there were little or no signs of postprandial toxicity.

"The animals were sacrificed on the 14th day and grossly examined at autopsy. The striking features noted were a light-colored liver having a nutmeg appearance of fat infiltration. The spleens were markedly congested and almost black. Again it was noted that the described pathology was more severe in the males than females although present in all. In the males there was apparent atrophy of the seminal vesicles with normal appearing testes. All other tissues were grossly normal. . . .

"Thus, sub-lethal doses of 500 mg/kg daily for 10 days is not lethal to mice, indicating little or no cumulative properties; however, apparent gross liver pathology observed at autopsy would indicate chronic toxicity."

From these results becomes evident that the animals have developed tolerance to the toxic effects of CPT (cyanosis, ataxia, coma), but there is no indication as to whether or not a tolerance to the lethal dose of CPT could also develop. It is not known if the lesions reported are reversible, and to what extent they may affect the health of the animal.

In a more recent preliminary study of tolerance development it was noticed that the rats receiving CPT consumed less food and water and that their rate of development was appreciably decreased. However, it was not elucidated if, beside this anorectic effect, CPT was affecting growth by some other mechanism also.

The present study was undertaken to examine: if the tolerance to the toxic effects of CPT could be developed in the rat; if a tolerance to lethal doses of CPT could also be developed; whether or not lesions possibly found in these species were persistent, their effect on the general condition of the animals, and finally if the retardation in the growth of the animals was due uniquely to the reduced feeding or to other toxic effects of CPT as well. For these purposes CPT was administered at progressively increasing doses to rats. In order to determine if anorexia was the only mechanism by which CPT was affecting growth, a group of rats were allowed food and water at amounts equal to those consumed by the rats receiving CPT.

Materials and Methods. Eighteen male rats of the Sprague–Dawley strain (Simonsen Laboratories Inc., Gilroy, California) weighing 100–120 g each were used in three groups of six: Group I, receiving CPT; Group II, receiving restricted diet but no CPT; and Group III, controls. Male animals were chosen because, according to the aforementioned study, male mice were more affected than females. They were kept in standard stainless-steel cages, at the usual laboratory temperature (23–26°) and fed with commercial feed (Purina Laboratory Chow). CPT hydro-

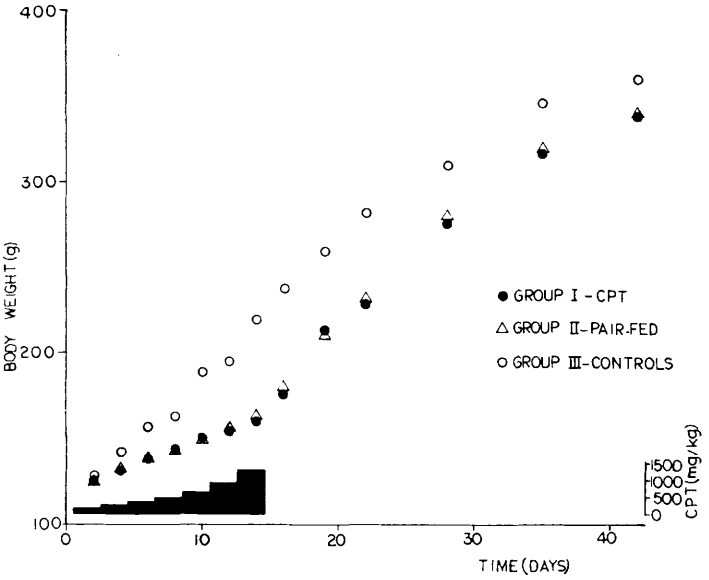

FIG. 1. Effect of 3-chloro-*p*-toluidine (CPT) on growth of rats. Each point is the mean of six rats. Standard deviations are not indicated because they did not exceed 9 g at any point. Group I. CPT daily for 14 days at doses increasing by a factor of 1.4 every second day, as indicated on the graph by black bars (initial dose 172 and final 1310 mg/kg of body weight); food and water *ad libitum*. Group II. No CPT; food and water at amounts equal to those consumed by Group I. Group III. Controls, food and water *ad libitum*.

chloride (purity 97%) was supplied by the U.S. Bureau of Sport Fisheries and Wildlife, Denver, Colorado. It was dissolved in tap water and administered into the stomach with the use of an intubation needle (Aloe Scientific VM 7 1042) connected to a syringe. Regardless of the dose administered, the volume injected was between 1.0 and 1.5 cm^3.

To the rats of Group I, CPT was administered daily for 14 days. The starting dose was 172 mg/kg of body weight and it was increased every second day by a factor of 1.4. Its LD_{50} was reported to be 655 mg/kg of body weight (1). The schedule for CPT administration is shown in Table I. To the animals of Group II and III 1.5 cm^3 of tap water was administered daily by intubation. Group I and III were allowed food and water *ad libitum* while Group II, during the administration period, received daily food and water at amounts equal to those consumed by Group I the previous day. The body weight of all animals was taken every second day. At the end of the administration period (Day 15) two rats from each group were sacrificed and subjected to autopsy. The remaining animals were under observation for 4 weeks post-treatment. At the end of this period they were sacrificed and subjected to autopsy.

TABLE I. Schedule for CPT Dosage.

Day of administration	Dose (mg/kg)
1 and 2	172
3 and 4	240
5 and 6	340
7 and 8	480
9 and 10	670
11 and 12	930
13 and 14	1310

Results. None of the animals of the three groups has presented any signs of acute toxicity. In the animals of Group I a barely distinguishable cyanosis was noticed (methemoglobin levels were not determined). In this group the most conspicuous sign was a reduction in water and food consumption (roughly ¾ of that of Group III) and a significant retardation in the rate of growth of the animals (Fig. 1). The same quantitative retardation was noticed in Group II (Fig. 1). Thus, the body weight of these 12 animals, at the end of the period of administration was

between 150 and 175 g (mean: 160 g) versus 180 to 250 g (mean: 220 g) of the animals of Group III; that is 73% of the body weight of the latter. At the end of this period, examination at autopsy of the two rats of Group I revealed a light-colored liver, having a nutmeg appearance of fat infiltration, and congested spleens. No lesions were found in four rats taken from Groups II and III. After discontinuation of the administration of CPT, the eight remaining rats in Groups I and II regained weight (Fig. 1) at such rapid rate as to reach 94% of the body weight of the control animals (Group III) in 4 weeks. During the post-treatment observation, no withdrawal or delayed effects of toxicity were seen. At autopsy, at the end of this period, no gross lesions were detected in the organs of the abdominal and thoracic cavities, or the brain.

Discussion. The results obtained in this study indicate that the animals have rapidly developed a tolerance to both the toxic and lethal effects of CPT.

The lesions found at the end of the administration period agree with those observed in mice (3). Their disappearance after 4 weeks indicates that they are transient and relatively rapidly reversible.

The retardation in growth during the administration period and the increase of weight being quantitatively the same in Groups I and II, it may be assumed that: the decrease in the rate of growth was not a direct and specific effect of CPT, and that no severe damage was caused by this rather massive administration of the avicide.

Summary. The avicide 3-chloro-*p*-toluidine (CPT, DRC-1339) was administered daily for 14 days to rats in doses progressively increasing from sublethal to twice the LD_{50}. No signs of acute toxicity were observed during this period or during the 4 weeks post-treatment. The only significant adverse effect was a retardation in the rate of their growth. The animals rapidly regain weight upon discontinuation of CPT administration. At autopsy some reversible lesions were observed in the liver and spleen. It appears that no serious permanent deterioration of the condition of the animals was caused by these massive doses of CPT.

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